

SPOILAGE OF TROPICAL FISH AND PRODUCT DEVELOPMENT



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

SPOILAGE OF TROPICAL FISH AND PRODUCT DEVELOPMENT

Proceedings
of a
Symposium held in conjunction with
the Sixth Session of the
Indo-Pacific Fishery Commission
Working Party on Fish Technology and Marketing

Royal Melbourne Institute of Technology
Melbourne, Australia, 23-26 October 1984

Edited by
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London

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PREPARATION OF THIS DOCUMENT

The supplement to the report of the Sixth Session of the Indo-Pacific Fishery Commission (IPFC) Working Party on Fish Technology and Marketing contains the papers contributed to the Symposium which have been edited by A. Reilly of the Tropical Development and Research Institute.

ABSTRACT

This publication contains the fifty-six papers of a Symposium on Spoilage of Tropical Fish and Product Development held in conjunction with the Sixth Session of the Indo-Pacific Fishery Commission (IPFC) Working Party on Fish Technology and Marketing at the Royal Melbourne Institute of Technology, Australia, 23-26 October 1984. These papers are divided into the ten following categories: Storage studies in ice and with delays in icing; Storage studies at elevated temperature; Frozen storage studies; Hygiene and seafood quality; Processed seafoods; Dried and salted fish; Histamine toxicity; Fermented fishery products; Product development and Progress Reports.

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QUALITY CHANGES AND BACTERIAL FLORA ASSOCIATED
WITH TRENCH SARDINES (*Amblygaster sirm*)
UNDER DELAYED ICING CONDITIONS

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ABSTRACT

When trench sardines (*Amblygaster sirm*), obtained under semi-commercial conditions, were iced immediately (0 h) or 5 h at ambient, the sensory, chemical and microbiological parameters 24 h after landing were not significantly different. However, if icing was delayed by 10 h after landing the fish, even though iced, was unsuitable for further processing. Twenty four hours after landing, total bacterial counts at 30°C from surface and flesh/gut ranged from 10^4 - 10^5 /g for 0 and 5-h sardines and 10^5 - 10^6 /g for 10-h sardines. Microflora of about 5% Gram negatives, consisted of predominantly *Pseudomonas* (41%), *Micrococcus* (10%) and *Bacillus* (28%) for trench sardines iced immediately on landing. If icing was delayed by 5 h, the proportions changed to *Pseudomonas* (14%), *Micrococcus* (43%) and *Bacillus* (14%).

1. INTRODUCTION

The total marine catch in Sri Lanka amounts to 185 506 t, of which around one third of the catch was clupeids-sardines, treach sardines and others. Therefore, maximum utilization of clupeids is of obvious advantage.

Trench sardines (*Amblygaster sirm*) will be one of the major raw materials for the retort pouching plant to be set up in Anuradhapura in Sri Lanka shortly.

However, varieties of small fish, obtained at most landing sites, tend to deteriorate rapidly if not suitably handled and iced. The short shelf lives of certain uniced tropical fish render them unsuitable for further processing, especially if not properly handled, iced and stored. Similarly, Villedsen *et al.* (1978) observed that silverbelly (*Leiognathus* sp.) deteriorated in only 6-8 h at ambient temperature (28°C) and Kamassatri, Sadanand and Rao (1967) recorded that silver pomfret had also spoiled after 8 h at ambient.

Several workers using inoculated sterile muscle blocks have associated spoilage characteristics, such as TVN and TMA production, "off odours" of fish with the activity of specific microflora isolated from spoiling fish. For example, Lerke *et al.* (1965), using sterile press muscle juice of English sole, showed that *Pseudomonas* sp. belonging to groups 1, 2 and 4 (Shewan, Hobbs and Hodgkiss, 1961), *Aeromonas* sp. and *Vibrio* sp. were active spoilers at 5°C. Herbert *et al.* (1971) showed that 13 strains of *Pseudomonas* and *Alteromonas* sp. were active in producing hydrogen sulphide and other sulphides in stale iced cod.

Very little research has been carried out on the flora of tropical fish and their effect on delayed icing. The present work examined the quality of raw materials (trench sardines), obtained from landing sites and thus its suitability for further processing.

2. MATERIALS AND METHODS

Fish samples were obtained from a landing site in Neganbo in Sri Lanka around 8:00 a.m., the usual time for most boats to reach the site. The fish obtained was divided into three lots (75 fish/lot). One lot was washed lightly in fresh water and iced at the site (0 h), the other two were transported uniced to fish boxes to the laboratory. One of the iced batches was iced after 5 h, whereas the other two batches were iced after 10 h of landing. On icing all samples were stored in a wet fish cabinet until around 8:00 a.m. the next day, when samples were withdrawn at random for visual, chemical and microbiological analyses.

The present study was repeated on three consecutive weeks by obtaining fish at the same landing site around the same time. All the fish were in juvenile stage of maturity.

2.1 Microbiological Analysis

Total bacterial counts (at incubation temperatures of 20° and 30°C) were estimated by plating serial dilutions of fish samples, prepared by homogenizing (in a Colworth Stomacher) 10 g of fish muscle obtained through the flush/gut regions, in 90 ml of sterile peptone (0.1% w/v) diluent. Surface plating techniques were employed to obtain a total bacterial count. Each of the dominating colonies from the surface were re-streaked onto nutrient agar (oxid). Once pure colonies were obtained, they (the colonies) were transferred onto NA slopes and stored for biochemical identification.

Surface counts were obtained by plating a (5 cm x 1 cm) sterile template on the surface of the fish and swabbing the area enclosed by the template. After swabbing three different fish, the swabs were transferred into peptone water (10 ml) and shaken well before aliquots (0.03 ml) of suitable dilutions were plated. Major representative colonies were stocked on NA slopes as mentioned earlier (for flush/gut sampling). Biochemical characterization of bacterial colonies was carried out according to the schemes of Shewan, Hobbs and Hodgkiss (1961) and Lee and Pfeifer (1975).

2.2 Chemical Analysis

Fifty grammes of fish fillet was minced well to a smooth paste, of which 25 g was used for total volatile nitrogen and trimethylamine determination (Anon., 1977).

The pH of fish/distilled water macerates (used for TVN) was measured using a radio meter 26 pH. Proximate analysis was also carried out (Anon., 1977).

2.3 Sensory Evaluation

Fish were evaluated by two-four panelists for texture, odour, sheen and condition of eyes and gills.

3. RESULTS

As given in Table 1, fish iced at site retained their original freshness after 24 h in ice. The odour was fresh and seaweedy with no loss in outer appearance or sheen. Slight softening of texture of some fish was observed, this characteristic being present in some fish even at time of landing.

Fish iced 5 h after landing retained most of the characteristics mentioned above. Consistent results were obtained for three trials as when compared with 0-h fish. However, variations in TVN and total bacterial count (TBC) were observed in trial 4, 0 h and 5 h showed no off odours, while a mild fishy odour was present in the 5-h fish.

Fish iced 10 h after landing was putrid and sulphidic, when observed after 24 h in ice storage. Loss of original translucency of the body was accompanied by reddening of the anterior and posterior regions.

Oil content of trench sardines (*Amblygaster sirm*) ranged from 1.2% to 2.2%, irrespective of icing treatments (Tables 2 and 3). The moisture content was around 74% (Table 2). Protein content of the fish ranged from a minimum of 22.3% to a maximum of 23.3% (Tables 2 and 4).

Total volatile nitrogen for fish iced at site ranged from 22.5 to 39.9 mg N/100 g, while for fish iced 5 h after landing values ranged from 30.6 to 47.6 mg N/100 g. However, TVN values of fish iced 10 h after landing increased to levels ranging from 71.4 to 114.2 mg N/100 g (Table 5).

The pH of trench sardines varied from 5.8 to 5.96 if iced on landing, while for fish iced 5 h after landing it ranged from 5.93 to 6.22. Similarly for fish iced 10 h after landing pH ranged from 6.27 to 6.60 (Table 6).

Total bacterial count (TBC) at 30°C of flesh/gut region was 10⁴/g, whether trench sardines had been iced at the landing site (0 h) or after landing (5 h) for two trials. Whereas in sampling 3 and 4, TBC was at 10⁴-10⁵/g, 1-1½ log cycles higher in fish iced 5 h after landing than if iced at site (0 h). In all four trials, the total bacterial count was 1-1½ log cycles higher in fish iced 10 h after landing than fish iced after a delay of only 5 h (Figure 1).

The total bacterial count was higher by 1½ log cycle when plates of 0-h and 5-h fish were incubated at 20°C rather than 30°C. However, incubation at 30°C had no effect on the total bacterial counts of 10-h fish (Figure 2).

Table 7 shows that the percentage of Gram negative and Gram positive organisms present in fish stored in ice for 24 h was 59% and 41%, respectively. Delayed icing up to 10 h had no effect on

the distribution of Gram negative and Gram positive organisms because even after 10 h, 54% of the isolates tested were Gram negative.

Of the fish iced at site, 41% of the flora consisted of *Pseudomonas*, which showed a decrease to 14% and 12% in fish iced 5 h and 10 h, respectively, after landing (Table 8). Although *Enterobacteriaceae* was not isolated from fish at site (Table 9), *Enterobacteriaceae* constituted 27% and 14% of the microflora of 10 h and 5 h fish treatments, respectively. Trench sardines with 5-h delay in icing were dominated by *Micrococci* (43%), which also constituted 23% and 10% of the flora of 10 h and 5 h fish treatments, respectively.

4. DISCUSSION

The overall quality of trench sardines approximately 24 h after catch, based on TVN content, TBC and visual characteristics, indicated that there is little difference between the quality of fish iced at the landing site and 5 h after landing. Several studies showed that the shelf life of some Sri Lankan fish was not affected by a delay in icing. Jayaweera *et al.* (1980) found that silverbelly (*Letognathus* sp.), iced at 1½ h and 4 h after landing, had a shelf life of eight days. In another study, silverbelly had a shelf life of 13 days whether icing was delayed by 3½ h or 6 h after landing. Similarly, scorapaw (*Selas leptocephalus*), whether iced 9 h or 12 h after landing, resulted in a shelf life of eight days (De Silva *et al.*, 1978); a shelf life of 21 days was recorded for flying fish iced either immediately or after 5 h at ambient (Chinivasagam and Goonwardene, 1979).

In the present study trench sardines iced after 10 h showed signs of deterioration and were unsuitable for further processing. In the four trials, the total bacterial counts of fish iced after 10 h at ambient (28°C) were 1 log cycle higher, at 10⁶/g than fish iced earlier. However, the total bacterial number does not indicate the number or type of individual organisms present (Castell, Anderson and Pivnick, 1948) and therefore is not regarded as a usable spoilage indicator.

Shewan (1977) states that bacteria are a function of the environment where warmer fish seem to have a more mesophilic Gram positive microorganisms (*Micrococci*, *Bacillus*, *Coryneformae*) than cold-water fish. Microflora of fish iced at the landing site in the present study and stored for 24 h in ice consisted of 43% Gram positives and 57% Gram negatives. Matanabe (1966) similarly isolated 46% to 48% Gram positives from Kariba bream stored for 24 h in ice.

Gram positives in trench sardines consisted of *Micrococci* (10%) and *Bacillus* (28%). Similar microflora, that is *Micrococci* (43%) and *Bacillus* (14%) was isolated from fish iced after 5-h delay at ambient. Wood (1953), Gillespie and MacRae (1975) found that warmer waters around Australia mainly consisted of *Micrococci*, *Bacillus* and *Coryneformae*. Venkataraman and Greenivasan (1952) obtained 18%, 55% and 19% *Bacillus* spp. from gills, slime and whole Indian mackerel, respectively.

In the present study, the Gram negative flora was dominated by *Pseudomonas* and *Enterobacteriaceae*. Fish iced at the landing site consisted of 41% *Pseudomonas* while fish iced after 5-h delay consisted of 14% *Pseudomonas* and *Enterobacteriaceae*. *Pseudomonas* species, mainly *Alteromonas putrefaciens*, have been incriminated as the main spoilers of fish in ice (Chai *et al.*, 1968) mainly due to the short generation time on ice (Shewan, 1977). Furthermore, *Alteromonas putrefaciens* are biochemically active, producing hydrogen sulphide, other volatile sulphides and reducing trimethylamine oxide (Gorczyca, 1983).

The low number of *Pseudomonas* in fish iced after 5-h delay at ambient (14% compared with 41% for fish iced immediately) may explain why delaying icing by 5 h had no effect on fish quality, the remaining microflora of 5-h fish being dominated by Gram positives, particularly *Micrococci*, at 43%.

Another possible reason for lower rate of spoilage on delayed icing of fish was suggested by Foulter, Curran and Disney (1981). He suggested that when warm-water fish are kept at high temperatures, the onset of rigor is slow and of a longer duration, while when kept on ice it is of a shorter duration as in roach, thus resulting in no bacterial growth till rigor is resolved.

Even though samples were obtained through flesh/gut region, the occurrence of *Vibrio* species was very low, unlike the observations recorded by Okuzumi and Horie (1969) and Yoshimizu, Kimura and Sakai (1976) in gut and intestines of fish. Whereas Liston and Baross (1973) suggest that *Vibrio* occurred in the Indian Ocean, the microflora of the intestines of fish was largely determined by the flora of the environment and food (Shewan, 1977), thus, possibly explaining why the *Vibrio* species were isolated in only low numbers in the present study.

Total volatile nitrogen, increasing during storage acted as a suitable index of spoilage in trench sardines. However, while initial values ranging from 22 to 39 mg N/100 g flesh were compared with 40 mg N/100 g as the limit of acceptability for cod, as proposed by Connel (1980).

The information discussed above suggested the possibility of using trench sardines as obtained under semi-commercial conditions from landing sites for further processing, provided the fish are iced, handled and stored satisfactorily, noting that a slight delay in icing period may not have a marked effect on the fish.

5. REFERENCES

- Cestell, C.W., G.W. Anderson and H. Pivnick, Relation of bacteriel counts to quality in cod fillets.
1968 J.Fish.Res.Board Can., 25(5):921-33
- Chai, T., et al., Detection and incidence of specific spoilage bacteria on fish. 2. Relative incidence of *Pseudomonas putrefaciens* and fluorescent *Pseudomonas* on haddock fillets.
1968 Appl.Microbiol., 16:1738-44
- Chinivasagam, N.H. and I.S.R. Goonawardene, Storage life of immediately iced and 5 hours delayed flying fish. Wet Fish IFT Sri Lanka (12). (internal report)
- Connell, J.J., Control of fish quality. Fernham, Surrey, England, Fishing News (Books) Ltd.,
1980 240 p. 2nd ed.
- De Silva, G.T.K., et al., Storage life of Soore purew (*Solas leptolepis*) with delayed icing of a
1978 9 and 12 hours. Wet Fish IFT Sri Lanka (6). (internal publication)
- Gillespie, N.C. and J.C. MacRae, The bacterial flore of some Queensland fish and its ability to
1975 cause spoilage. J.Appl.Bacteriol., 39:91-100
- Gorczyca, E.M., Studies on the shelf life extensions of retail fish fillets. M.Appl.Sci.Thesis,
1983 Applied Chemistry Department, RMIT, Vic 3001, Australia
- Herbert, R.A., et al., Bacterie active in the spoilege of certain seefoods. J.Appl.Bacteriol.,
1971 34:41-50
- Jayaweera, V., et al., Storage life of silverbelly (*Leiognathus* spp.) with delayed icing. Bull.
1980 Fish.Res.Stn., Sri Lanka, 30(1-2):53-61
- Kamasatri, P.V., V.G. Sedanand and R. Rao, Studies on the storage characteristics of silver pomfret
1967 (*Pampus argenteus*) transported to Bombay. Fish.Technol.Soc.Fish.Technol.,Ernakulam,
4:71-7
- Lee, J.S. and D.K. Pfeifer, Microflora associated with Dangersness crabs (*Cancer magister*). Appl.
1975 Microbiol., 30:70-3
- Lerke, P., R. Adams and L. Ferher, Bacteriology of spoilege of fish muscle. 3. Charecterisation
1965 of spoliars. Appl.Microbiol., 13:625-30
- Liston, J. and J. Baross, Distribution of *Vibrio parahaemolyticus* in the natural environment.
1973 J.Milk Food Technol., 36(2):113-7
- Okuzumi, M. and S. Morie, Studies on the bacterial flora in the intestines of various marine fish.
1969 Bull.Jap.Soc.Sci.Fish., 35:93-100
- Poulter, R.G., C.A. Curran and J.G. Disney, Chill storage of tropical and temperate water fish.
1981 Differences and similarities. Refrig.Sci.Technol., 4:111-23
- Shewan, J.M., The bacteriology of fresh and spoiling fish and the biochemical changes induced by
1977 bacterial action. In Proceedings of the Conference on handling, processing and
marketing of tropical fish. London, Tropical Products Institute, pp. 51-66
- Shewao, J.M., G. Hobbs and M. Hodgkiss, A determinative scheme for the identification of certain
1961 genera of bacterie with special reference to Pseudomonadeceae. J.Appl.Bacteriol.,
23:463-8
- Venkataraman, R. and A. Sreenivasan, A preliminary investigation of the bacteriel flore of the
1952 mackerels of the West coast. Indian J.Med., 40:529-33
- Villedsen, A., et al., Storage life of silverbelly with delayed icing of 1.5, 4, 8 and 12 hours.
1978 Wet Fish IFT Sri Lanka, (9)
- Watanabe, K., Handling and keeping quality of iced Karihe bream (*Filapia mortimeri*, Syn.
1966 *T. moresambica* Peters). Fish.Res.Bull.Zambia, 4:59-64

- Wood, E.F.J., Heterotrophic bacteria in marine environments of eastern Australia. Aust.J.Mar.
1983 Freshwat.Res., 4:161-97
- Yoshimizu, M., T. Kimura and M. Sakai, Studies on the intestinal microflora of salmonids. 1. The
1976 intestinal microflora of fish reared in freshwater and seawater. Bull.Jap.Soc.Sci.Fish.,
42:91-9
- Anon., A collection of analytical methods and testing procedures for the assessment of fish and
1977 shellfish quality. Paper presented at the CIDA/FAO/CECAF training course (TF INT 180(f)
CAN) on fish handling, plant simulation, quality control and fish inspection. Dakar,
Senegal, 10 October - 4 November 1977

Table 1

Observations of fish iced at sits 0 h, 5 h, 10 h after landing

Description	Visual observation after (h) in ice		
	0 h	5 h	10 h
<u>Sampling 1</u>			
Appearance	Shiny, good looking, eyes clear, gills red	Shiny, eyes clear, good looking, gills reddish	Red around head and tail regions, loss of shine, no slime, gills brown
Odour	Fresh/seaweedy	Fresh	Bad odour, mild H ₂ S
Texture	Firm	Fairly firm	Soft
<u>Sampling 2</u>			
Appearance	Shiny, good looking, eyes clear, gills red	Shiny, eyes clear, good looking, gills reddish	Dull and showing reddish stains on body, gills dull
Odour	Fresh/seaweedy	Fresh/fishy	Spoiling odour, putrid
<u>Sampling 3</u>			
Appearance	Shiny, good looking, eyes clear, gills slightly red	Shiny, eyes clear, gills red	Dull and showing reddish patches, gills dull red
Odour	Fresh/seaweedy	Fresh/fishy	Spoilt odour, putrid, slight H ₂ S
Texture	Firm	Fairly firm	Softening
<u>Sampling 4</u>			
Appearance	Shiny, good looking, eyes clear, gills red	Shiny, eyes slightly red	Dull, showing reddish patches in central and tail regions
Odour	Fresh/seaweedy	Fresh/good	Spoilt odour, putrid, slight H ₂ S
Texture	Firm	Fairly firm	Very soft

Table 2

Approximate (%) composition of *Amblygaster sirm*^{a/}

Protein	Dry matter	Oil
22.60	25.38	1.53

^{a/} Average of two results

Table 3

Oil content of *Amblygaster sirm*

Sampling	Oil content (%) after time (h) at ambient ^{a/}		
	0 h	5 h	10 h
1	1.56	1.20	1.43
2	1.52	1.98	2.21
3	1.28	1.56	1.63

^{a/} Average of two readings of a composite sample of 5 fish

Table 4

Protein content in *Amblygaster sirm*

Sampling	Protein content (%) after time (h) at ambient ^{a/}		
	0 h	5 h	10 h
1	22.61	22.49	23.34
2	22.45	22.36	-

^{a/} Average of two readings of a composite sample of 5 fish

Table 5

Effect of delayed icing on total volatile nitrogen in *Amblygaster sirm*

Sampling	Total volatile nitrogen (mg/100 g) after time (h) at ambient ^{a/}		
	0 h	5 h	10 h
1	22.54	30.54	71.45
2	25.67	44.66	114.23
3	32.09	41.15	73.18
4	39.89	47.61	-

^{a/} Total volatile nitrogen (TVN-N) as an average of 3 readings of a composite sample of 5 fish

Table 6

Effect of delayed icing on pH in
Amblygaster sirm

Sampling	pH after time (h) at ambient ^{a/}		
	0 h	5 h	10 h
1	5.8	6.10	6.60
2	5.91	6.22	-
3	5.87	5.93	5.87
4	5.96	6.01	6.27

^{a/} Average of two results of a composite sample of 5 fish

Table 7

Effect of delayed icing on the major grouping of
microflora

Gram stain	Numbers (%) of major representative colonies ^{a/} after time (h) at ambient		
	0 h	5 h	10 h
Gram negative	17(59)	15(43)	14(54)
Gram positive	12(41)	20(57)	12(46)

^{a/} Major representative colony types obtainable as a percentage of 4 platings, from a composite sample of 10 g, obtained from 5 fish, through flesh/gut and a surface swab of 5 cm² as an average of 4 consecutive samplings carried out on weekly basis

Table 8

Effect of delayed icing on the Gram positive microflora on *Amblygaster sim*

Microflora	Numbers (%) of colonies ^{a/} (major representatives) obtained after time (h) at ambient		
	0 h	5 h	10 h
<i>Micrococcus</i>	3(10)	15(43)	6(23)
<i>Bacillus</i>	8(28)	5(14)	5(19)
<i>Corenibacterium</i>	1 (3)	-	1 (4)
<i>Staphylococci</i>	1 (3)	-	1 (4)

^{a/} Major representative colony types obtainable as a percentage of 4 platings, from a composite sample of 10 g, obtained from 5 fish, through flesh/gut and a surface swab of 5 cm² carried out on weekly basis

Table 9

Effect of delayed icing on the Gram negative microflora on *Amblygaster sim*

Microflora	Numbers (%) of colonies ^{a/} (major representatives) obtained after time (h) at ambient		
	0 h	5 h	10 h
<i>Pseudomonas</i>	12(41)	5(14)	3(12)
<i>Acinetobacter</i>	2 (7)	3 (9)	2 (8)
<i>Enterobacterium</i>	-	5(14)	7(27)
<i>Flavobacterium</i>	2 (7)	-	-
<i>Vibrio</i>	-	2 (6)	-
<i>Vibrionaceae</i>	-	-	1 (4)
Unidentifiables	5	-	-

^{a/} Major representative colony types obtainable as a percentage of 4 platings, from a composite sample of 10 g, obtained from 5 fish, through flesh/gut and a surface swab of 5 cm² carried out on weekly basis

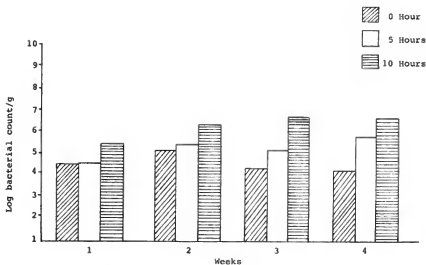


Figura 1 Changes in total bacterial count of *Amblygaster sive* stored in ice

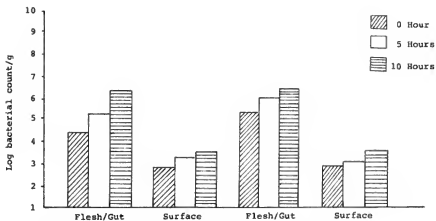


Figura 2 Total bacterial count on flesh/surface at 30°C and 20°C

QUALITY CHANGES IN BOLIVIAN FRESHWATER
FISH SPECIES DURING STORAGE IN ICE

by

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ABSTRACT

This study was made to determine the iced shelf life and spoilage pattern of selected commercial and semicommercial species of Bolivian freshwater fish. Established chemical, microbiological and sensorial methods of evaluation were used. Fish studied from the cold waters of the Altiplano region were: pejerrey (*Basilichthys bonariensis*) from Lake Poopo and Lake Angostura; carp (*Cyprinus carpio*) and trout (*Salmo gairdneri*). The shelf lives of these fish in crushed ice were 21, 15, 20 and 15-16 days respectively, based on the sensory evaluations. One fish species collected from the warmer low-land waters of the Parana basin, sabalo (*Prochilodus platensis*), was found to have a shelf life in ice of approximately 25 days, regardless of whether they had been stored whole or eviscerated. Larger fish from the warm Amazonian waters showed extended shelf lives in ice: pacu (*Colossoma macropomum*), chinchuna (*Pseudoplatystoma tigrinum*) and tambaqui (*Colossoma brachypomum*) all had shelf lives exceeding 40 days. Whilst other important commercial species exhibited shelf lives of 30 days corvina (*Plagioscion squameostictus*) and 19 days - bagre (*Acanthonotus* spp.). Several other fish species of less commercial value from Amazonian waters were also investigated and included: tachaca (*Piarodon granulosus*), seferino (*Hypophthalmus edentatus*), sardinon (*Pellona flavipinnis* and *P. castelnaui*) and blanquillo (*Pirapomus pirapomus*). Thus fish from all areas of Bolivia were found to have acceptable storage lives in crushed ice and this would therefore facilitate their distribution to urban centres distant from the major fishing location.

1. INTRODUCTION

Very few studies have been published on the storage characteristics of freshwater fish when kept in ice, in contrast to the wealth of information that is available for marine fish species. However, the small amount of information that is available on the iced storage characteristics of freshwater fish does indicate that patterns of spoilage are similar to those of marine species (Shewan, 1977), but that their storage lives generally appear to be longer (Bramstedt and Auerbach, 1961). In view of this and to enable distribution and marketing networks to be established a survey was conducted to determine the iced storage characteristics of a variety of Bolivian freshwater fish species from both temperate and tropical waters.

It is generally accepted that the storage lives of fish in ice are closely related to the temperature and type of environment from which they are caught. Thus, many species of tropical fish from warm waters have been reported to have storage lives in ice of 3 to 4 weeks compared with about 2 weeks for many species from cold temperate water. This difference has mostly been explained in terms of the types of microflora found on tropical fish. The suggestion is that the flora found on tropical species will be adapted to live at higher ambient temperatures and be mesophilic in nature, whereas the bacteria which cause spoilage of fish in ice are known to be psychrotrophic in nature (Disney, 1976; Shewan, 1977; Liston, 1979). Furthermore, it has been observed that certain physical and chemical characteristics, including the shape, size and fat contents, can all combine to influence the duration of iced storage (Disney, Cole and Jones, 1974; Shewan, 1977). The precise reasons for these observed differences in storage lives have been considered at length by research workers and various explanations have been put forward. Such explanations have included the possible antimicrobial properties of fish muscle and/or slime (Matanabe, 1966), the production of inhibitory substances by spoilage bacteria and low post-mortem pH of muscle which may inhibit bacterial growth (Shewan, 1977; Liston, 1979).

Bolivia has three main fisheries, each one being ecologically distinct. These are: 1) the glacial cold water lakes of the Altiplano; 2) the rivers Pilcomayo and Bermejo in the sub-tropical Parana basin to the south east of the country and 3) the tropical waters of the Amazon basin to the north east of Bolivia (Figure 1).

The predominant fish species caught in these areas are pejerrey from the Altiplano, sabalo from the Parana basin and pacu, tambaqui and the catfishes surubi and chincuína from the Amazon basin. The Amazon region has the greatest potential for fisheries development in Bolivia since many more fish species are abundant and available from this area.

1.1 Fish Studied

Species of fish collected for ice-storage studies were:

(1) Altiplano basin

- | | |
|--------------|--|
| (a) pejerrey | <i>Basilichthys bonariensis</i> |
| (b) trout | <i>Salmo gairdneri</i> (from a trout farm) |
| (c) carp | <i>Cyprinus carpio</i> |

These fish species were stored entire in crushed ice.

(2) Parana basin

- | | |
|------------|------------------------------|
| (e) sabalo | <i>Prochilodus platensis</i> |
|------------|------------------------------|

Sabalo was stored in ice both whole and eviscerated.

(3) Amazon basin

- | | |
|---------------|-----------------------------------|
| (a) pacu | <i>Colomesoma macropomum</i> |
| (b) tambaqui | <i>Colomesoma brachypomum</i> |
| (c) chincuína | <i>Pseudoplatystoma tigrinum</i> |
| (d) corvina | <i>Plagioscion squamosissimus</i> |
| (e) bagre | <i>Ageniatus</i> spp. |

In addition the proximate chemical composition of the muscle and observations on the changes of the physical characteristics of the following potentially commercial fish species stored in ice were made.

- | | |
|--------------|-------------------------------------|
| (a) tachaca | <i>Pterodorus granulatus</i> |
| (b) seferino | <i>Hypophthalmus edentatus</i> |
| (c) sardinao | <i>Pellona flavipinnis</i> |
| (d) machete | <i>Rhaphiodon vulpinus</i> |
| (e) curimate | <i>Curimata gestioetomus latior</i> |
| (f) eigménie | <i>Eigmannia</i> spp. |
| (g) pirane | <i>Serrasalminus naterri</i> |

Because of their size and in order to comply with traditional handling techniques, pacu, tambaqui and chincuína were all headed and gutted prior to storage in ice. Some fishermen store the smaller tambaqui whole and additional trial was conducted with such fish. Corvina, bagre and the potentially commercial fish species studied, which are all appreciably smaller, were stored whole. The results of this study are to be published shortly. A review of the information obtained is discussed below.

2. RESULTS AND DISCUSSION

2.1 Altiplano Fish

The basic data obtained for carcass evaluation and proximate chemical composition of freshly captured Altiplano fish are given in Table 1. Little difference in the weights and sizes of pejerrey caught from the two different lakes was found and they were all very small (overall average weight = 53.30 ± s.d. 2.02 g). The proximate composition of the muscle of these Altiplano fish was fairly similar in most respects. However, the pejerrey coming from Lake Poopo were found to have one of the highest muscle fat contents whilst pejerrey from Lake Angostura the lowest. Trout showed one of the

highest crude protein contents which may have been influenced by the type of artificial diet that they had received (Table 1).

The physical and sensory evaluations showed a gradual deterioration on storage of the fish in ice. Pejerrey from Lake Poopo and Angostura showed similar rates of decline in their G.R. Torrymeter readings with time throughout the duration of the trial (Figure 2). The taste panel results for general acceptability (Figure 3) also showed similar rates of deterioration for about the first 11 days, during which time the fish were in very good condition. Thereafter, however, the changes in the visual and olfactory characteristics of these two lots of fish differed significantly. Rupturing of the body cavity and liquefaction of gut contents (belly burst) were evident after only 8 days of storage with pejerrey from Lake Angostura, whilst belly burst did not occur with pejerrey from Lake Poopo. This particular physical deterioration could possibly have resulted in a more rapid decline in the sensory qualities of pejerrey from Lake Angostura (Figure 3). From the values obtained by taste panel and using the score of 4 as that value which indicates the fish to be just unacceptable, then maximum storage lives in ice for pejerreys from Lakes Angostura and Poopo differed significantly and would be 15 and 21 days, respectively (Table 3).

The use of the G.R. Torrymeter with carp from Lake Angostura provided readings which declined fairly smoothly with storage time (Figure 2). This was not, however, the case with the results of the sensory evaluations. The mean scores for the overall acceptability of fish remained high and in the range 6 to 7 for the first 15 days (Figure 3). At this time, like pejerrey from the same lake, these fish started to show signs of belly burst and this coincided with a rapid fall in acceptability scores. Using the acceptability scores as a guideline to maximum storage life then about 20 days were recorded.

The results of the G.R. Torrymeter with trout from Lake Titicaca differed from all of the other fish species studied from the Altiplano. It was observed that a decline in meter readings was only obtained for the first 8 days of storage (Figure 2). Thereafter, and for the rest of their storage life, mean readings remained constant ($6.04 \pm \text{s.d. } 0.56$). The converse, however, was true for the overall acceptability scores during storage (Figure 3). The trout showed very advanced signs of belly burst on and after 12 days of storage. The overall acceptability scores would indicate a maximum storage life for trout held in crushed ice of 15 to 16 days.

2.2 Bacteriological Assessments

The three species of fish investigated from this region showed initial bacterial loads of between 10^2 and 10^4 cfu/g of muscle on day 1 (Figure 4). These levels rose to exceed 10^7 cfu/g between 17-20 days of iced storage which is the maximum microbiological limit for fresh fish recommended by the International Commission of Microbiological Standards for Foods (ICMSF, 1978). Therefore, the results obtained indicated that the four species of Altiplano fish had storage lives in ice of between 17-20 days.

2.3 Chemical Analyses

The values obtained for the pH of the fish muscles rose with duration of storage (Figure 5). The initial pH of the muscle of most fish was in the range of 6.3-6.4. The values obtained for trout were exceptionally erratic and would not provide a useful index of fish freshness with this species.

The TVB content of Altiplano fish muscles indicate that all the fish had initial values of between 4-7 mg N/100 g (Figure 6). Connel, 1975 has suggested that for marine species of fish such as cod, TVB values of 30-40 mg N/100 g flesh may be taken as an indication that the fish has reached its limit of acceptability. In these investigations pejerrey from Lake Poopo were found never to reach these levels whilst pejerreys from Lake Angostura exceeded 30 mg N/100 g after 17 days. This result would seem to confirm the acceptability score as representing the more appropriate index of maximum storage life for this species. Nonetheless, it must be noted that the 30-40 mg N/100 g limit has been suggested for a marine fish species. However, freshwater fish may differ significantly from their salt water counterparts in that they often contain little or no trimethylamine oxide (TMAO) (Hebard, Flick and Martin, 1982). Trimethylamine, the degradation product of TMAO, was not studied in the present investigation.

Thus, dependent upon the index used, the storage lives of the pejerreys from different lakes were found to differ significantly (Table 3). However, the sensory characteristics of fish must in the final event determine whether the consumer accepts or rejects the product. Consequently, the maximum acceptable storage life for these particular fish must be taken to be 15 days.

2.4 Parana Basin Fish

2.4.1 Carcase evaluation and proximate chemical composition

Sabalo were found to have an average weight of just over 1 kg and when eviscerated lost a total of about 13% of their original weights (Tabla 2a). The percentage yield of skin-on-fillets recorded

from whole fish was 55.1%. Sabalo is regarded by most Bolivian consumers as being a "fatty fish". The proximate enclyses carried out upon the muscias of these fish, (Table 2a), do not fully confirm this assumption and they may be regarded as having a medium fat content. They were, however, found to have relatively high crude protein and low moisture contents.

However, large fat deposits were found to be associated with the gut tissue. During the breeding migration of this fish from the lowland swamps in Paraguay to the headwaters of this river they apparently do not feed. Undoubtedly, therefore, during their upstream mass migrations they utilize these body fat stores for energy and as a result their muscle fat contents are likely to fluctuate.

2.4.2 Physical and sensory evaluations

Sensory evaluations were not conducted with this fish but it was epperent from the visual and olfactory observations that at storage times in excess of about 25 days that the fish were of extremely poor quality. A previous study has indicated a maximum acceptable iced storage life for the sabalo from the same river, based on taste panel analysis, of slightly less than 24 days (R.R. Coutts, pers. comm., 1982).

The fish showed the usual appearance characteristics and olfactory qualities associated with fresh fish. On storage in ice these characteristics started to diminish after 5 to 6 days. There were few noticeable differences in the rates with which spoilage occurred between entire sabalo and eviscerated sabalo.

The G.R. Torrymeter readings obtained with this fish species declined gradually and fairly linearly over the period of the storage trial (Figure 7) and the technique would appear to provide a useful index of fish freshness. The effects of evisceration upon the meter readings and their rates of decline appeared to be minimal (Figura 7).

2.4.3 Bacteriological assessments

No notable differences in the absolute values or rates of increases in bacterial numbers were observed between fish stored whole or eviscerated (Figure 7). The results for sabalo when compared with those increases in bacterial numbers found for Altiplano fish (Figure 4) show more gradual increases resulting to curves with considerably reduced gradients. This reduced rate of bacterial growth indicates that psychrotrophic bacteria may not be present in very high numbers during the initial stages of storage.

2.4.4 Chemical analyses

The values for pH obtained for both whole and eviscerated sabalo increased with length of storage to become close to 7 after about 25 days (Figure 7). Eviscerated fish showed appreciably lower initial values than fish which had been stored entire. However, these differences were reduced with length of storage and were minimal after about 17 days.

The TVB content of muscle from sabalo were found to be very low initially ranging from 2.64-2.85 mg N/100 g (Figure 7). On storage of both whole and eviscerated fish these values increased very slightly and after about 10 days were in the range 10-15 mg N/100 g. After 32 days of storage the final TVB content of whole and eviscerated sabalo were found to be 26 and 45 mg N/100 g, respectively.

2.5 Amazon Fish

2.5.1 Carcass evaluation and proximate chemical composition

The three fish which form the basis of the commercial fishery in this region of Bolivia were pacu, tambaqui and the catfish known locally as chincuína. The fish were all large and showed ranges in average weights of 3.9 to 15.5 kg and average total lengths of 57 to 1,172 cm (Table 2a). The removal of head, gills and guts from these fish was found to result in losses of total weight of between 24.0% (chincuína) and 30.3% (pacu) (Table 2a). The greatest contribution to these losses came from the heads. In all these fish fat glands were found to be associated with the gut tissue and were often retained by fishermen for rendering into oil. The muscles themselves also showed very high fat contents ranging from 8.85% (chincuína) to 18.02% (Table 2a). The two semi-commercial and the potentially commercial species collected from the Amazon were all smaller and had lower fat contents (Tables 2a and 2b).

2.5.2 Physical and sensory evaluations

It was noticeable with the fish stored headed and gutted that the skin and body cavity started to be coated with large quantities of yellow malodorous slime after about 16 days. At this time the belly flaps and other tissue which had become exposed during heading and gutting became very soft,

almost liquefied. The scales of the pacu and tambaqui were by the end of the trial loosened and the black markings of chinchua could be easily rubbed off. Similar changes were also found for the smaller fish species investigated, although the changes were found to occur more rapidly. With these smaller fish, which were all stored whole, the eyes were found to become cloudy and sunken quite rapidly (5 days) and the gills changed in colour from being red through pink or brown to white. The changes described above could be used to form the basis of a fish inspection system if required.

The results of the G.R. Torrymeter evaluations are given in Figure 2. It was found for chinchua, a catfish with a reasonably high fat content, that the meter readings fell very rapidly during the first five days of storage, from values of 11-12 to 2-3, and thereafter declined more slowly over the remaining storage period to zero. The fish species corvina and bagre showed more linear declines in meter readings over the storage periods; values falling from being in the range 11-12 to 1-2 over 25 days. The two large and scaled commercial fish, pacu and tambaqui, which are species of the same genus *Colomesoma* spp., showed similar changes in their G.R. Torrymeter readings during storage (Fig. 2). The method of storage of tambaqui, namely whole or headed and gutted, appeared to have little effect on the rates of changes of Torrymeter readings.

Interestingly, the scores obtained from sensory evaluations over the period of the trials did not fall rapidly. For example, the case of both whole and gutted tambaqui the average scores remained in the range of 6.2-8.0 for the total period of the 38-day trial (Fig. 3). These scores, therefore, never indicated that these fish were unacceptable to taste panelists. It appeared, therefore, that the fish were still acceptable despite the very low G.R. Torrymeter readings and extremely poor visual and olfactory characteristics found near the end of the trials. A similar result was found for pacu, chinchua and corvina although with these fish an initial decline over the first 10 to 15 days was apparent followed by a plateau with scores in the region of 5-7.

2.5.3 Bacteriological assessment

The large and commercial species; pacu and tambaqui showed signs of bacterial invasion after only 2 days of iced storage. At this time the muscles of pacu were found to have 10^3 cfu/g while tambaqui had values of 10^4 cfu/g (Figure 4). The bacterial load of these large fish were found to increase steadily with storage time in ice and had reached about 10^7 cfu/g muscle after 30-36 days. There appeared to be no obvious differences between tambaqui stored entire or headed and gutted in their rates of spoilage and their maximum storage lives using ICMSF recommended bacteriological guidelines (ICMSF, 1978). Storage periods were determined to be 35-36 days. This level (10^7 cfu/g) however, was more rapidly reached by the small, non-commercial fish species studied. Thus, both corvina and bagre were found to have maximum storage lives as determined using this bacteriological guideline of approximately 25 days.

2.5.4 Chemical analyses

The initial pH values obtained for all the fish studied were in the range 6.1 and 6.25 (Figure 5). On subsequent storage the pH values of chinchua, corvina and bagre all increased; more rapidly for the first 20 days or so and then levelling out at around pH 7 for the remainder of the trials. Pacu and tambaqui, however, showed an initial fall in pH between days 2 and 10. This was followed by a smooth gradual rise in pH for the remainder of the storage period and after 38 days they had all reached pH 7. Both whole and headed/gutted tambaqui showed similar initial declines and subsequent increases in pH on storage.

The fish had initial TVB values in the range of 4-7 mg N/100 g. These levels increased gradually for the following 15-20 days of storage and then more rapidly for the remaining period. Tambaqui, both in the whole and headed/gutted form showed similar relative changes and after 83 days of iced storage had values exceeding 75 mg N/100 g. Headed and gutted pacu, however, showed less rapid increases in volatile base content and after the same period of storage only contained about 34 mg N/100 g (Figure 6).

Storage lives in ice given in Table 3 which indicate the larger Amazon fish species to have storage lives of about 40 days confirms claims made by fishermen for the longevity of their produce. Certainly, from sensory evaluations the fish are still acceptable after about 40 days. However, all other indicators of acceptability, most particularly bacterial numbers point to significantly shorter storage lives. It is possible that the inadequate use, if any, of ice, the poor knowledge of basic hygiene and lack of facilities used by fishermen, distributors and in the market places have resulted in the consumer, by necessity, becoming accustomed to buying and eating poor quality fish. Clearly, fish of over 40 days storage, though still acceptable to consumers may carry a certain risk. For example, the high levels of bacteria present may produce toxins, or contribute towards the formation of other toxic chemical constituents such as histamine.

The assessments for sensory characteristics conducted in this investigation do perhaps represent one of the more relevant methods for the determination of fish quality since it is on the basis of such evaluations that the consumer ultimately accepts or rejects the product. They do not, however, provide an unequivocal measure of quality since by definition they depend on the senses and adjustments of individuals and, therefore, be subject to personal prejudices and likes. This bias was clearly seen in the present investigation with fish from the Amazon. The results showed that many of the fish were still highly acceptable, for over 40 days despite poor visual and olfactory characteristics and high bacterial contamination. Possible reasons for such results were mentioned earlier in this discussion but it is noteworthy here, since the question arises of whether fish should be considered to have reached their maximum storage lives when, (a) the consumers reject the fish or (b) when other physical, chemical and bacteriological analyses indicate rejection. Such a question cannot be easily answered in a country such as Bolivia in which food is often limited and where American and European bases of quality and hygiene have no real grounding. Should option (a) be taken as the most appropriate index of storage life then it must be made clear to all concerned that should the analyses conducted in option (b) indicate earlier rejection that such indications have been made for reasons of their increased potential toxicity. Consequently, the vendor and consumer must be made aware of the possible increased health hazard and implications of preparing and consuming such fish.

3. CONCLUSIONS

The fish studied in this investigation ranged very widely in their shapes, sizes, weights and chemical compositions. In general it may be summarized that fish from the cold waters of the Altiplano had low fat contents and were small whilst fish from warmer waters of the lowland rivers, were larger and had much higher fat contents. It is also evident that fish from the warmer lowland rivers of the Parana basin (River Pilcomayo) and the Amazon (Rivers Mamore and Secure) had considerable longer storage lives in ice than fish from the colder waters of the high Altiplano lakes.

However, apart from a clear environmental effect upon the length of storage of the different fish species, their size played a significant role. Thus for example, the smaller tropical fish species collected from the Amazon, such as corvina and bagre, and sabalo from the River Pilcomayo, showed significantly shorter maximum storage lives than larger species of fish collected from the same area. It should be noted that the fish from the Altiplano were all very small, even when compared with these smaller fish species collected from lowland locations. The principle that has already been demonstrated by other studies (Disney, 1976; Shewen, 1977) where indications are given that fish from tropical warm waters tend to have longer shelf lives in ice than similar types of fish from temperate cold waters is consistent with the results of this study (Table 6).

The occurrence of belly burst amongst the Altiplano fish studied caused a decline in taste panel scores. It is advised that fish such as pejerrey, carp and trout be stored in ice eviscerated. Conversely sabalo and tambaqui showed few noticeable differences whether stored entire or eviscerated.

The G.R. Torrymeter has been developed and tested extensively with cold water species and has been found to give quick, reliable indication of the quality of fresh fish. In recent years several studies have been conducted to determine its use with tropical fish and these have been reviewed by Poulter and Curran, 1982. The use of the meter in this investigation was found to provide a useful index of fish quality with many of the species studied. This was so with pejerrey and carp from the Altiplano, sabalo from the River Pilcomayo and pecu, tambaqui and corvina and bagre from the Amazon. However, the use of the G.R. Torrymeter with a species of catfish from the Amazon, chincupina, was not of any value since the readings declined rapidly during the first few days of storage. The possible reasons for the poor results obtained with the meter on these fish may be related to their thick and leathery skins which are often associated with large deposits of underlying adipose tissue. These catfish were also found to have high muscle fat contents. All these factors are known to affect the readings made by the Torrymeter (Poulter and Curran, 1982).

In conclusion, therefore, the determination of visual and olfactory characteristics in conjunction with taste panel evaluations could provide an adequate system for the assessment of freshness and quality of all the fish species. It would also provide rapid back up information to support and monitor these assessments. Results have indicated that Total Volatile Bases and pH values could not be used on their own to give any accurate assessment of the quality of fish. The application of bacteriological assessments has been shown to be of greatest value from these studies but the obvious delay in obtaining results, due to times, would mean that such analyses could not be used for routine screening.

This study provides a review of the storage lives and quality characteristics of Bolivia's fish species. As such, the study will provide basic information of vital importance to future projects concerned with fisheries development in Bolivia. It is hoped that this will enable optimum distribution and marketing networks for fresh fish to be established.

4. REFERENCES

- Bramstedt, T.F. and M Auerbach, The spoilage of freshwater fish. In Fish as food, edited by 1961 G. Borgstrom. New York, Academic Press, vol. 1:613-7
- Connell, J.J., Control of fish quality. Farnham, Surrey, England, Fishing News (Books) Ltd., 179 p. 1975
- Disney, J.G., The spoilage of fish in the tropics. In Proceedings of the First annual tropical 1976 fisheries technological conference. College Station Texas, Texas AandM University, pp. 23-39.
- Disney, J.G., R.C. Cole and N.R. Jones, Considerations in the use of tropical species. In Fishery 1974 products, edited by R. Kreuzer. Surrey, England, Fishing (Books) Ltd., for FAO, pp. 329-37
- Hebard, C.E., G.J. Flick and R.E. Martin, Occurrence and significance of trimethylamine oxide and 1982 its derivatives in fish and shellfish. In Chemistry and biochemistry of marine food products, edited by R.E. Martin, *et al.* Westport, Connecticut AVI Publishing Co., pp. 149-304
- ICMSF, (International Commission of Microbiological Standards for Foods), Microorganisms in foods, 2. 1976 Sampling for microbial analysis. Principles and specific applications, edited by the International Commission on Microbiological Specifications for Foods. Toronto, Canada, University of Toronto Press, pp. 92-104
- Liston, J., Microbiology in fishery science. In Advances in fish science and technology, edited by 1979 J.J. Connell. Farnham, Surrey, England, Fishing News (Books) Ltd., pp. 138-57.
- Poulter R.G. and C.A. Curran., Use of the G.R. Torrymeter with tropical species of fish. Paper 1982 presented to the Symposium on Harvest and post-harvest technology of fish. Chocin, India, Society of Fisheries Technologists, 11 p. (mimeo).
- Shewan, J. M., The bacteriology of fresh spoiling fish and the biochemical changes induced by 1977 bacterial action. In Proceedings of the Conference on the handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 511-66.
- Watanabe, K., Handling and keeping quality of iced kariba bream, *Tilapia mortimeri*, Trewavas. (Syn. 1966 *T. mosambica*, Peters). Fish.Res.Bull., Zambia (4): 54-64.

Table 1

Basic data for freshly captured Altiplano fish species used in iced storage trials

Fish Species	PEJERREY	PEJERREY	CARP	TROUT
Fishing Location	L. Angostura Cochabamba	L. Poopo Oruro	L. Angostura Cochabamba	L. Titicaca La Paz
Average Total Weight	55.32g	51.27g	132.06g	90.13g
Range	32.5-77.5	27.8-93.5	70.8-198.1	63.2- 122.9
Average Stand. Length (cm)	16.85	17.04	17.53	17.38
Range	13.6-19.1	15.0-21.7	14.2-22.1	15.4-19.5
Average Total Length (cm)	19.91	20.74	21.97	20.25
Range	17.0-24.3	18.0-26.1	18.0-27.5	18.2-23.0
Average Torrymeter (Day 0)	13.22	11.10	13.06	13.59
Range	10-16	8-12	12-14	13-14
Carcase Analysis (%)				
Fillets	55.2	56.5	49.9	63.0
Muscle	47.7	47.1	45.2	53.4
Skin	7.7	9.6	4.7	9.6
Head	21.4	18.0	20.5	11.6
Skeleton	10.6	13.4	16.3	11.8
Viscerae	7.9	7.6	8.5	10.4
Gills	4.7	4.3	4.8	3.2
Proximate Analysis of Muscle (%)				
Moisture	80.00	79.70	81.64	76.50
Protein (N x 6.25)	17.32	17.86	15.95	20.52
Fat	0.65	3.62	2.09	3.51
Ash	0.76	0.78	0.92	1.06

Tabla 2a

Basic data for freshly captured Paraná Basin and Amazonian fish species used in feed storage trials

Fish Species	SABALO	PACU	TAMBAQUI	CHINCUÍÑA	CORVINA	BACRÉ
Fishing location	R. Pilcomayo Tarija	R. Securé Beni	R. Securé Beni	R. Securé Beni	R. Memoré Beni	R. Memoré Beni
Average total weight Range	1.016-00 g 600-1950	8.93 g 7.2-12.5	3.88 kg 2.0-6.0	14.53 kg 13.7-15.3	2017 g 1000-1025	669 kg 337-1025
Average stand length (cm) Range	34.54 26.4-42.4	66.54 46-74	52.63 47-58	1015 975-1150	36.36 29.5-44	26.63 21-32
Average Total length (cm) Range	42.44 36.4-52.0	75.21 57-87	57.31 48-66	1172 1152-1191	50.71 43-60	37.13 42-32
Average Torrymeter (Day 0) Range	11.00 10-12	11.57 10-12	10.81 9-13	11.80 10-13	12.11 10-13	10.83 10-12
Carcass Analysis (%)						
Fillate	55.1	52.3	48.4	-	56.5	52.02
Muscle	45.4	44.2	39.4	50.5	49.5	48.7
Skin	7.7	8.10	9.3	10.5	7.0	7.3
Head	17.7	20.7	16.6	11.7	23.8	30.1
Skeleton	16.5	17.4	16.6	15.2	11.3	10.9
Viscerae	10.3	5.3	7.3	7.8	5.9	4.0
Gills	3.2	4.3	2.2	4.5	2.6	3.1
Proximate Analysis of Flesh (%)						
Moisture	67.00	67.09	69.27	70.75	67.89	78.98
Protein (N x 6.25)	23.38	14.11	15.84	18.89	21.69	14.77
Fat	4.26	18.02	15.61	8.85	5.90	3.66
Ash	1.46	0.86	0.98	1.01	0.77	0.53

Table 2b
Basic data for freshly captured Amazonian fish species used in iced storage trials

Fish species	SEPERINO	SAEDINON	MACHETE	CURIMATA	EIGMANNIA SP.	PIRANHA
Fishing location	R. Memoré Beni	R. Memoré Beni	R. Memoré Beni	R. Memoré Beni	R. Memoré Beni	R. Memoré Beni
Average total weight Range	675 g 510-710	12454 g 972-1328	1191 g 775-1400	51.54 g 22-110	89.29 g 70-140	95.23 g 73.3-117.3
Average stand length (cm) Range	46.54 39.1-56.2	29.8 27.1-30.2	46.8 33.4-56.1	12.65 9.6-16.8	18.95 17.2-22.3	13.12 12.9-16.50
Average Total length (cm) Range	56.53 44.5-61.5	33.4 29.3-35.4	51.5 37.1-68.7	13.77 10.5-17.5	21.29 19.0-24.5	15.33 13.8-16.91
Average Torrymatar (Day 0) Range	11.33 10-13	12.20 11-13	12.36 12-13	14.13 13-15	13.00 13	13.33 12-14
Carcan Analysis (g)						
Filleta	56.3	39.0	38.4	48.6	65.1	-
Muscle	47.4	32.1	27.1	39.4	51.5	39.3
Skin	8.9	7.3	11.6	9.0	13.7	8.0
Head	11.1	19.5	13.4	20.8	16.1	23.8
Skalaton	26.7	26.8	31.7	18.8	9.9	16.4
Viscetas	3.7	4.9	3.7	6.5	4.4	9.3
Gills	3.0	4.9	1.2	5.5	4.5	3.2
Proximate Analysis of Fish (g)						
Moisture	66.36	77.47	70.47	83.07	75.75	-
Protein (N x 6.25)	18.20	16.18	15.05	16.93	18.05	-
Fat	17.24	2.99	13.39	0.92	6.66	-
Ash	0.71	1.04	0.85	0.70	0.59	-

Table 3

Maximum storage lives of fish species studied as determined by sensory evaluations (taste panel acceptability scores) and bacteriological assessments

Fish Species		Maximum Storage Life (Days)	
		Sensory Evaluations	Bacteriological Assessments
Pejerrey (E)	- L. Poopo	21	20
Pejerrey (E)	- L. Angostura	15	18-19
Carp (E)	- L. Angostura	20	17-18
Trout (E)	- L. Titicaca	15-16	17-18
Sabalo (E)	- R. Pilcomayo	-	25
Sabalo (EV)	- R. Pilcomayo	-	25
Pacu (HG)	- R. Securé	40	36
Chinquíana (HG)	- R. Securé	40	30-31
Tambaquí (E)	- R. Securé	40	33-34
Tambaquí (HG)	- R. Securé	40	35-36
Corvina (E)	- R. Mamoré	30	25
Bagre (E)	- R. Mamoré	19	25

E = Entire EV = Eviscerated HG = Headed and Gutted.

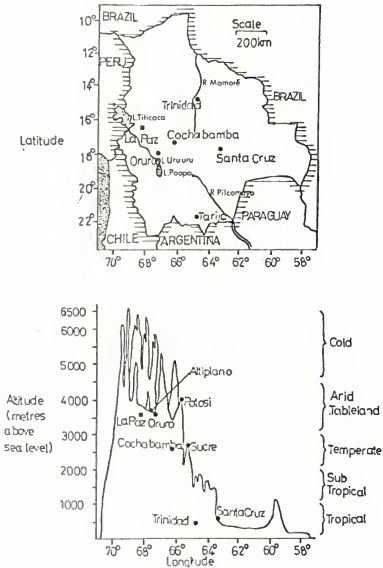
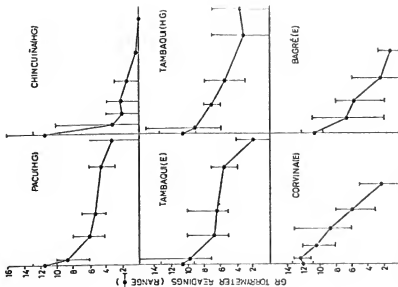


Figure 1 Map and longitudinal profile of Bolivia

AMAZONIAN FISH



ALTIPLANO FISH

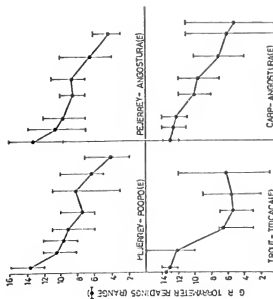
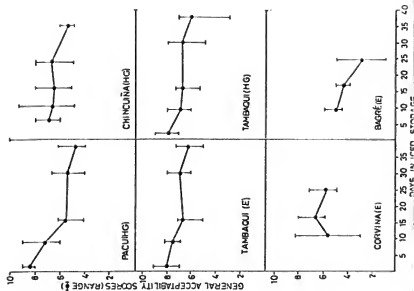


Figure 2 GR Torrymeter readings of Altiplano and Amazonian fish stored in ice

AMAZONIAN FISH



ALTIPLANO FISH

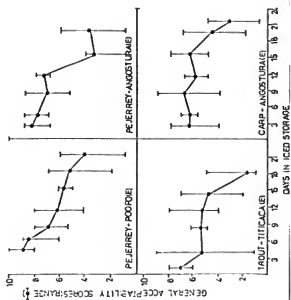


Figure 3 Sensory evaluation (general acceptability scores) of Altiplano and Amazonian fish stored in ice

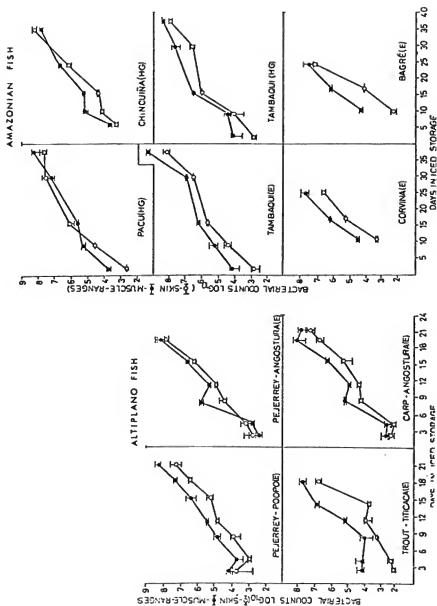


Figure 4 Bacterial counts at 25°C of Altiplano and Amazonian fish stored in ice

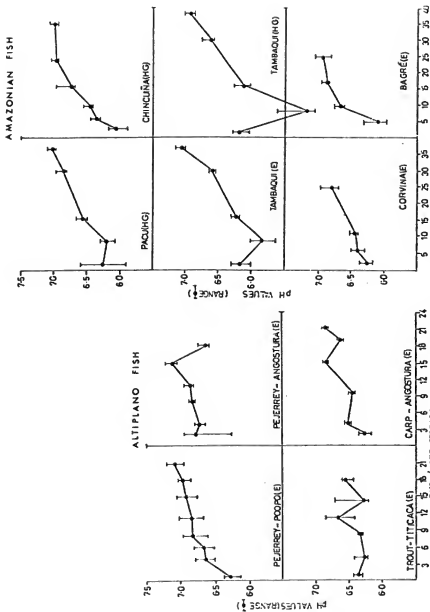


Figure 5 pH of Altiplano and Amazonian fish stored in ice

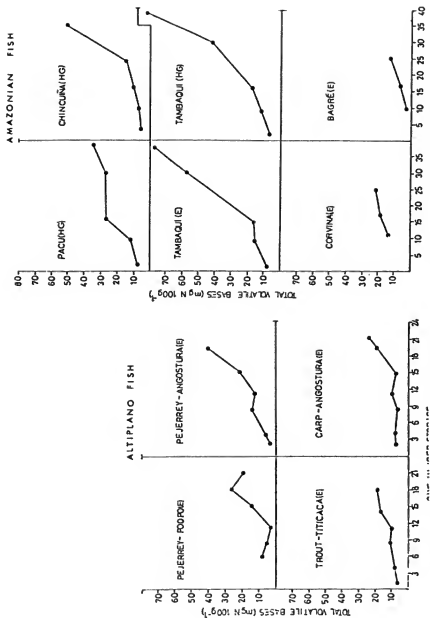


Figura 6 Total Volatile Bases content of muscle of Altiplano and Amazonian fish stored in ice

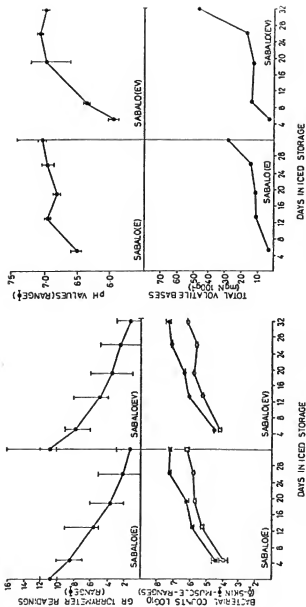


Figure 7 GR Torrymeter readings, bacterial counts, pH values and Total Volatile Bases of pilcomayian fish

SPOILAGE PATTERNS OF MACKEREL (*Rastrelliger faughni* Matsui)

1. DELAYS IN ICING

by

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ABSTRACT

Shelf life of Faughn's mackerel in ice was reduced by one day for every hour delay in icing or exposure to ambient temperature of 28° to 30°C. Storage at ambient temperature for 12 h resulted in spoilage after an additional 7-h storage in ice. Nucleotide decomposition expressed as K-values were 56-71% when fish were spoiled. Hypoxanthine ranged from 1.08 µM/g at rejection for samples immediately iced to 1.33 µM/g for 12 h delay in icing. Both tryptamine and total volatile nitrogen levels fluctuated throughout storage and were of questionable value as quality indices for this species. The standard plate count (20°C) on rejection by a trained taste panel decreased from 10⁹/g at 0-h to 10⁷/g after 3-h delay, 10⁶/g after 6-h delay and 10⁵/g after 9-h and 12-h delay. The principal spoilage organisms after 0-6 h delay in icing were *Pseudomonas* spp. and *Alteromonas putrefaciens*. Prolonged delay before icing (9-12 h) gave a different final spoilage flora of *Bacillus*, *Aeromonas hydrophila* and *Pseudomonas* spp.

1. INTRODUCTION

Most commercial fishing boats operating in Lamon Bay, off the West Coast of the Philippines are purse-seiners which remain at sea for less than one day, while small-scale, low-income fishermen spend about 4-6 h at sea. Fish caught by both commercial and small-scale fishermen are generally not iced on board and if ice is used, the ratio of fish to ice is insufficient to chill the catch. Fish are not iced before landing because ice is too expensive for the low-income groups or it is unavailable in rural fishing communities. On arrival at landing sites fish have been exposed to high ambient tropical temperatures which lead to rapid quality deterioration. Few studies exist on the spoilage patterns of tropical fish with delayed icing. Only limited information is available on the spoilage microflora of tropical fish exposed to ambient temperatures. Identification of the microflora, especially the fish spoilers, may be of value in predicting the shelf life.

This study investigated the effects of 3, 6, 9 and 12-h delay in icing after catching on the overall quality of Faughn's mackerel (*Rastrelliger faughni* Matsui), and determined the length of time small-scale fishermen can stay at sea, without ice, before there is a serious decrease in quality of this species.

2. METHODS

2.1 Sampling

Two storage trials were carried out on Faughn's mackerel caught by commercial purse-seiners in Lamon Bay where the water temperature was between 26°-27°C and air temperature 28°C.

Immediately after sorting the catch according to species, Faughn's mackerel (locally known as *alumahan*) were divided into 5 lots. Most of the fish were alive when iced. The four other lots were kept in insulated fish boxes at ambient temperatures (28°-30°C) for 3, 6, 9 and 12 h, then chilled in a 1:1 ice to fish ratio. All samples were brought back to the laboratory of the University of the Philippines in the Visayas (UPV), College of Fisheries where samples were stored in a chill room at 5°C until the end of the experiment to reduce temperature fluctuations. Facilities and conditions on board commercial fishing vessels made it impossible to conduct analyses

immediately after catching. Samples for chemical and proximate analyses were immediately frozen in dry ice while samples for initial microbiological tests were placed in sterile plastic bags, sealed and iced. Delayed icing samples were similarly stored and analysed with 24 h of catching. Storage conditions were maintained by draining the insulated fish boxes of melted ice and more ice added to keep the temperature at 0°C throughout the entire storage period.

Sensory, chemical and microbiological analyses were carried out at intervals of 2-3 days to determine the overall quality, according to the sample scheme outlined in Figure 1.

Fish Samples

Stored in ice, 1:1 ice to fish ratio
after 3, 6, 9 and 12 h delayed icing

Forty-four pieces were taken each
sampling day for the different tests.
Samples for microbiological analysis
were sampled aseptically.

Eight fish were used for microbiological and chemical tests, 36 fish for sensory; 3 raw and 3 cooked samples presented to each taste panel member.

<u>Chemical Tests</u>	<u>Microbiological Tests</u>	<u>Sensory Tests</u>
1. K-value	1. Standard plate count at 20°C	1. Raw samples (whole) odour, general appearance, texture, overall accept- ability
2. Hypoxanthine	2. H ₂ S producers count at 20°C	2. Cooked samples (Gutted & boiled in 2% brine for 10 minutes) odour flavour, texture, overall accept- ability
3. TMA/TVN	3. Identification of spoilage microflora	
4. Proximate composition		

Figure 1 Sampling scheme

2.2 Sensory Assessment

A descriptive scorecard was used by six trained taste panelists to evaluate the raw and cooked sensory attributes using a 10 to 0 hedonic scale (Figures 2 and 3). Panelists were also requested to indicate the frequency and degree of belly burst in the raw samples as traditionally Faughn's mackerel is not gutted during storage. Raw samples were rinsed under the tap and presented whole to the taste panel. Cooked samples were prepared by boiling whole gutted mackerel in 2X brine for 10 min. Shelf life was based on the cooked flavour score of 4 as the limit of acceptability.

2.3 Chemical Analyses

Moisture, protein, fat and ash content of Faughn's mackerel were determined by the methods of the AOAC (1975).

Total volatile nitrogen (TVN) and trimethylamine (TMA): Trichloroacetic acid extracts were prepared from 30 g of fish flesh and TVN and TMA determined by the Conway microdiffusion technique (Conway, 1968).

Hypoxanthine (Hx): Hx content was determined on neutralized perchloric acid extracts according to the method of Burt, Murray and Stroud (1968). K-value, which is expressed as the percentage ratio of Hx and inosine (HxR) to the total amount of ATP-related compounds was estimated by the method of Kobayashi and Uchiyama (1980).

2.4 Microbiological Analyses

The standard plate count (SPC) and hydrogen sulphide producers were determined on peptone iron agar (Jensen and Schultz, 1980), after incubation at 20°C for 72 h. Twenty (20) colonies were randomly isolated for each SPC and identified according to the methods of Cowan (1974) and MacFaddin (1980). Confirmatory tests for gram negative bacteria were carried out using the API20E system (API International, 65 Rue de la Prulay, 1217 Meyrin, Geneva, Switzerland).

3. RESULTS AND DISCUSSIONS

The results on the proximate composition of Faughn's mackerel are presented in Table 1.

All sensory attributes on raw and cooked samples of Faughn's mackerel gave significant correlation with days storage at 1% level. Rejection of raw samples by trained taste panelists was mainly characterized by rancid to strong rancid, sour and fishy odours and very soft texture. The general appearance was rated below the acceptable limit based on sunken, opaque eyes with yellowish, reddish and/or slightly greenish cornea and faded characteristic colour of the skin with brownish discoloration on the ventral part of the body. Cooked samples gave sulfidic, sour and rancid odours. The predominant flavours were rancidity and bitterness with biting aftertaste. Cooked texture was judged as dry and fibrous. Shelf life based on cooked flavour score of 4 shows that for every hour delay in icing an equivalent one day storage in ice is lost.

Regression analyses (Figure 4) illustrate that the rate of decline in flavour scores as delays in icing are extended results in no great loss of cooked flavour when fish are maintained for up to 6 h at ambient temperature, but the effective storage life in ice was reduced. Fish held at ambient temperature for 12 h spoil after 7 h when stored in ice. Flavour changes were found to be 26.6 times faster at ambient temperature than at 0°C. This is reflected in the shorter shelf lives after delaying icing for 9 and 12 h. After storage at ambient temperature for more than 6 h fish began to lose their sweet flavour, which developed as bland and rancid. These changes are more pronounced in samples held for 9 and 12 h before icing. Similar results were reported for silurabelly (*Leiognathus* sp.) where the main spoilage began after 6-8 h at 28-30°C (Jayaweera *et al.*, 1980).

The incidence of belly bursting in the different samples and its effect on the rate of quality deterioration were taken into account. Strong belly bursting took place earlier in samples with prolonged exposure to ambient temperatures (Figure 5). This defect has been attributed to digestive enzymes present in the contents of the gut (Gildberg and Rao, 1980).

Changes in initial flavour qualities of Faughn's mackerel with delays in icing was accompanied by an increase in the rate of nucleotide degradation as expressed by the K-value, which is defined as the concentrations inosine (HxR) + hypoxanthine (Hx) over the total nucleotide degradation products ATP, ADP, IMP, HxR and Hx times 100 (Ehira, 1976). The loss of freshness expressed by K-values was 37.5 times faster at ambient temperature than at 0°C. An increase in initial K-values was found as delays in icing were extended. Values increased from 6.0% for immediately iced samples to 69.6% for 12 h samples. Significant correlation at 1% level was found between K-values and days in ice for all samples (Figure 6), with rejection limits of 56, 57, 66, 61 and 71% for 0, 3, 6, 9 and 12 h delay in icing respectively. Very fresh fish have a K-value of less than 20% and reach the point of incipient spoilage at values above 60% (Ehira and Uchiyama, 1974).

DATE :

Instructions : Choose the description from the scale that best characterizes the samples presented to you. Place the corresponding score/mark for the said description under the column for each sample code. Additional comments will be well appreciated.

1	2	3	4	5	6	7	8	9	10
TYPE : SHAPE									
Completely snake	Snake	Slightly snake	Fist	Follow the contour of the body	Curves				
COLOR OF PUPILS									
Completely opaque	Opaque	Slightly opaque	Gray to slightly opaque	Still black (grayish)	Bright, clear black				
COLOR OF CORNEA									
Blackish/ yellow/ overgreen	Strong yellow/ red or green	Yellow/ red and/ or slightly greenish	Less red and/ or slightly greenish	Less transparent pale-rose or reddish	Transparent, slight discoloration				
COLOR OF SKELETON									
Pale/white greenish	Pale (pinkish)	Pale brown	Red-rose	Dark red (brownish)	Dark red bright red				
COLOR/CONDITION OF MOUTH									
As outside	Thin watery red-rose	Thin red-rose reddish	Reddish brown	Thick reddish	Thin transparent				
SIZE									
Character- teristic outline mostly faded	Character- teristic outline faded/ brownish-yellow along dorsal, opercular & pectoral regions	Character- teristic outline slightly faded	Character- teristic outline slightly faded	Character- teristic outline slightly faded	Yellowish along lateral line/ pale yellowish along dorsal/ lateral line to ventral				
GENERAL APPEARANCE									

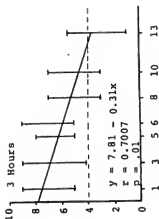
Note : Belly burst is not considered when rating the general appearance of the samples.
Put a check () mark across the word that best describes the degree of belly burst.
Each check () mark represents one fish, two checks for two fish with belly burst, etc.

1	2	3	4	5	6	7	8	9	10
COLOUR									
Strong purple/brassy	Strong purple	Weakish	Slightly weakish	Neutral slightly weakish	Fresh and/or oily				
PUPILS									
Pupils prominent	Spotted strong fishy	Weak	Slightly weak	Neutral to slightly fishy	Fresh sweetly				
TEXTURE									
Very soft	Soft	Soft and elastic	Less elastic	Firm and elastic	Rigid, very firm and elastic				
GENERAL ACCEPTABILITY									

Figure 2 Scorecard for chilled alumnin (Rastrelliger faughni Matsui) - Raw samples

NAME :		DATE :						
Instructions : Choose the description from the scale that best characterizes the samples presented to you. Place the corresponding figure/word for the said description under the column for each sample code. Additional comments will be well appreciated.								
	3	4	5	6	7	8	9	10
ODOUR								
Strong rancid		Rancid (sour)	Slightly rancid	Little rancid	Slightly rancid	Slightly sweet	Fresh sweet	Fresh sweet
Spilled		Sour & fishy	Slightly fishy	Little fishy	Slightly fishy	Slightly sweet	Fresh sweet	Fresh sweet
Ammoniacal								
Strong sulphid								
FLAVOUR								
Strong		Rancid	Slightly rancid	Little rancid	Slightly rancid	Slightly sweet	Fresh sweet	Fresh sweet
Strong bitter/		Slightly bitter	Slightly bitter	Trace of "itchy"	Slightly bitter	Slightly sweet	Fresh sweet	Fresh sweet
Strong								
Putrid		Strong	Slightly strong	Little strong	Slightly strong	Slightly sweet	Fresh sweet	Fresh sweet
Stale		Strong	Slightly strong	Little strong	Slightly strong	Slightly sweet	Fresh sweet	Fresh sweet
Stale (itchy)		Strong	Slightly strong	Little strong	Slightly strong	Slightly sweet	Fresh sweet	Fresh sweet
TEXTURE								
Dry & gritty		Dry & gritty	Slightly dry	Little dry	Slightly dry	Slightly firm	Firm	Firm
Soft & mushy		Soft & mushy	Slightly soft	Little soft	Slightly soft	Slightly firm	Firm	Firm
Very soft		Very soft	Slightly very soft	Little very soft	Slightly very soft	Slightly very soft	Very soft	Very soft
Very mushy		Very mushy	Slightly very mushy	Little very mushy	Slightly very mushy	Slightly very mushy	Very mushy	Very mushy
GENERAL ACCEPTABILITY								
0	1	2	3	4	5	6	7	8

Figure 3 Scorecard for chilled alumahan (*Rastrelliger faughni* Matsui) - Cooked samples



days

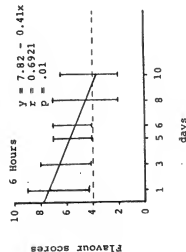
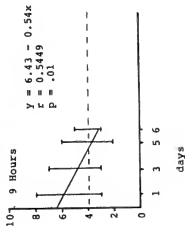


Figure 4 Correlation between cooked flavour scores and storage time with delays in icing (Vertical lines indicate the range of taste panel scores)

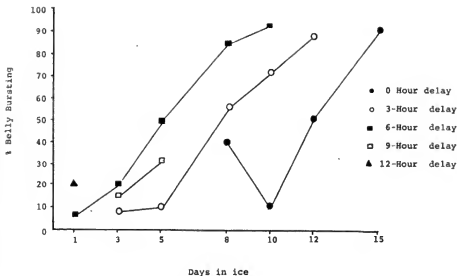


Figure 5 Incidence of belly bursting in Faughn's mackerel with delays in iced storage

Faughn's mackerel can be stored for 3-4 h at ambient temperature and still be considered fresh according to these limits. The rejection levels for K-values also agree with the above limits, except for the 12-h delay in icing where the level was higher.

Initial Hx levels for all samples increased with delays in icing. Storing fish at ambient temperature for 12 h resulted in an increase from 0.18-1.0 $\mu\text{H/g}$. Rejection values were between 1.08-1.33 $\mu\text{H/g}$ (Table 2). Only immediately iced samples gave increasing Hx levels with storage time. Significant correlation at 12 level existed between Hx end time of storage for samples with 0 and 3-h delay, however further delays in icing resulted in poor correlation.

TMA and TVN values were found to fluctuate after delayed icing and subsequent storage in ice (Table 3). However, regression analysis showed significant correlation between storage time and TMA content only for fish immediately iced after catching ($r = 0.97$; $p = 0.01$). No correlation was found for TVN levels and storage time or delay in icing. Both TMA and TVN are of questionable use as indices of spoilage for Faughn's mackerel.

The changes in SPC's, H_2S producers counts and percentage H_2S producing bacteria of the total count are presented in Figure 7. The initial values were 10^4 , 10^5 and $10^6/\text{g}$ for 0, 6 and 12-h delay respectively. No initial bacterial count was recorded for 3 and 9-h delay due to contamination of the agar plates. However, bacterial numbers increased when kept at ambient temperature for up to 12 h. Prolonged delays prior to icing resulted in a decrease in rejection level after iced storage on account of the washing effect of melting ice and a decrease in the number of mesophiles. On rejection SPC's were $10^9/\text{g}$ for fish immediately iced, $10^8/\text{g}$ for 3 h, $10^6/\text{g}$ for 6 h, $10^5/\text{g}$ for 9 and 12 h delay in icing. The H_2S producers accounted for 4-8% of the total counts for all samples on rejection. The results for fish immediately iced and 3-h delay samples agree with those of Liston (1982), in that H_2S producers count exceed $10^6/\text{g}$ at rejection. It is not possible to establish this rejection level in terms of numbers of H_2S producers for samples held at ambient temperature for 6, 9 and 12 h.

The changes in bacterial flora during iced storage and delays in icing are shown in Figure 8. The initial load was predominantly mesophilic in nature and comprised of *Bacillus* spp. (40%); *Pseudomonas* spp. (30%); *Acinetobacter*, *Corynebacterium* and *V. enterocolitica* at 10% respectively. When samples were iced immediately the numbers of *Pseudomonas* spp. and *Alteromonas putrefaciens* gradually increased with storage time and accounted for 95% or $8.6 \times 10^8/\text{g}$ on rejection. After delaying icing for 3 and 6 h similar results were obtained where *Pseudomonas* spp. and *A. putrefaciens* made up 89

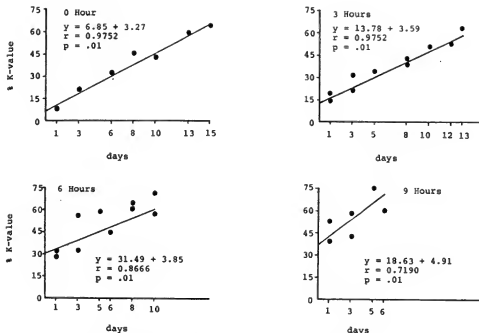


Figure 6 Correlation between % K-value and storage time after delays in icing

Table 1

Proximate composition of Faughn's mackerel

Fish part	% Moisture	% Protein	% Fat	% Ash
Whole	74.2	13.97	1.66	3.14
Flesh	75.4	17.61	0.56	1.32

and 94% of the spoilage flora respectively. Storage at ambient temperature for 9 h prior to icing gave rise to an increase in the number of mesophiles which predominated the bacterial load after one day in ice (Table 4). When rejected by the taste panel the bacterial load comprised *Bacillus* spp. (36%), *Pseudomonas* spp. (22%) and *Aeromonas hydrophila* (21%). A similar increase in mesophiles was found for samples kept for 12 h at ambient temperature and had a storage life of 7 h in ice.

Table 2

Hypoxanthine (Hx) content ($\mu\text{M/g}$) of Faughn's mackerel stored in ice after 0, 3, 6, 9 and 12 h at ambient temperature

Days in ice	Hypoxanthine (Hx) $\mu\text{M/g}$				
	0 Hour	3 Hours	6 Hours	9 Hours	12 Hours
0	0.16	0.21	0.50	0.81	1.00
1	0.20	0.28	0.57	0.93	1.33
3	0.35	0.20	0.42	0.73	
6	0.28	0.49	0.86	1.22	
8	0.57	0.49	1.22		
10	0.64	0.93	1.15		
13	0.49	1.15			
15	1.08				

Table 3

Trimethylamine (TMA) and Total Volatile Nitrogen (TVN) (mg/100 g) content of Faughn's mackerel stored in ice after 0, 3, 6, 9 and 12 h at ambient temperature

Days in ice	TMA/TVN (mg/100 g)									
	0 Hours		3 Hours		6 Hours		9 Hours		12 Hours	
	TMA	TVN	TMA	TVN	TMA	TVN	TMA	TVN	TMA	TVN
1	0.18	19.02	0.64	21.91	0.79	18.00	1.65	20.41	2.20	21.73
3	0.28	15.13	0.27	16.09	0.55	16.93	0.89	19.33		
6	0.31	12.61	0.31	9.09	0.79	14.71	0.91	15.91		
8	0.36	13.63	0.39	14.23	0.75	15.13				
10	0.47	14.71	0.44	16.45	0.57	14.41				
13	0.63	18.61	0.66	27.0						
15	0.79	31.93								

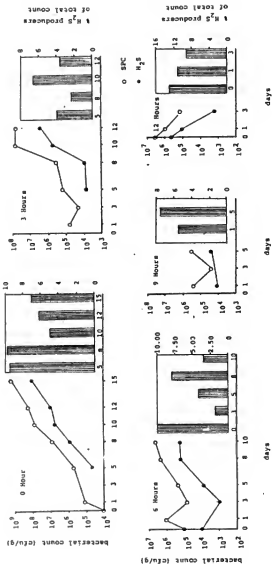


Figure 7 Standard plate count at 20°C, H₂S producers count at 20°C and % H₂S producers of Faughm's mackerel with 0, 3, 6, 9 and 12-hour delay in icing and subsequent storage in ice

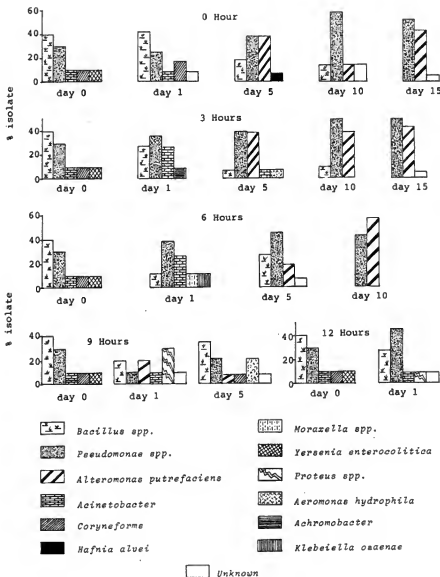


Figure 8 Changes in the bacterial flora (X) of Faughn's mackerel with 0, 3, 6, 9 and 12-hour delay in icing and its subsequent storage in ice

4. REFERENCES

- ADAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1975 ADAC, 12th ed.
- Burt, J.R., J. Murray and G.D. Stroud, An improved automated analysis of hypoxanthine. J. Food Technol., 3:165-70
- Conway, E.J., Microdiffusion analysis and volumetric error. London, Crosby, Lockwood and Son, 1968 467 p.
- Cowan, S.T., Cowan and Steel's manual for the identification of medical bacteria. Cambridge, 1974 Cambridge University Press, 238 p.
- Ehira, S., A biochemical study on the freshness of fish. Bull. Tokai Reg. Fish. Res. Lab., (88):1-128 1976
- Ehira, S. and H. Uchiyama, Freshness-lowering rates of cod and sea bream viewed from changes in bacterial count, TVB and TMA-Nitrogen and ATP related compounds. Bull. Jap. Soc. Sci. Fish., (40):479-87
- Gildberg, A. and J. Raa, Tissue degradation and bally bursting in capelin. In Advances in fish science and technology, edited by J.J. Connell. Surrey, England, Fishing News (Books) Ltd., pp. 253-8
- Jayaweesa, V., et al., Storage life of silverbally (*Leiognathus* sp.) with delayed icing. Bull. Fish. Res. Stn., Sri Lanka, (30):53-61
- Jensen, M.H. and E. Schultz, Utilization of iron agar in determining the freshness of wet fish. Dan. Vet. Tidsskr., 63:314-8
- Kobayashi, H. and H. Uchiyama, Simple and rapid method for estimating the freshness of fish. Bull. Tokai Reg. Fish. Res. Lab., 61:21-6
- Liston, J., Recent advances in the chemistry of iced fish spoilage. In Chemistry and biochemistry of marine products, edited by R.E. Martin et al., Westport, Connecticut, AVI Publishing Co., 27-38

TROPICAL SPECIES FROM THE NORTH-WEST SHELF OF AUSTRALIA:
SENSORY ASSESSMENT AND ACCEPTABILITY OF FISH STORED ON ICE

by

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ABSTRACT

Four tropical species from the North-west Shelf of Australia were obtained during a cruise of the CSIRO Division of Fisheries Research vessel, the FRV SOELA in November 1983. The four species *Nemipterus peronii* (threadfin bream), *Argyrops spinifer* (longspine snapper), *Plectorhynchus pictus* (painted sweetlip) and *Lutjanus vittatus*, a snapper-like fish that has no commercial name, were either frozen on board post-rigor or iced and then sent by air-freight to the CSIRO Division of Food Research, Tasmanian Food Research Unit, Hobart. The appearance and odour of the fish during storage on ice was evaluated using a sensory assessment scheme based on systematic allotment of merit points for defects as they occurred during the storage period. Stained flesh from each species was assessed organoleptically at each sampling time using a profile taste panel. After about three weeks storage, samples of iced fish were frozen, then at a later date fillets from them and from fish that had been frozen on board were deep fried and assessed by taste panel for their acceptability. The sensory assessment scores and the profile panel indicated a marked deterioration in the iced fish over the three-week storage period. The deep fried fillets from the stored fish were, however, judged to be acceptable.

1. INTRODUCTION

The Northwest Shelf area of Australia has been fished commercially by Japanese trawlers from 1958 to 1963 and by Taiwanese pair trawlers and gillnet boats from 1971 to the present (Sainsbury, 1979). The area is highly productive, yielding an annual catch of wet fish greater than that caught in the rest of Australia. Australian fishermen have not exploited the resources of the Northwest Shelf and to date there have been no published studies on the intrinsic properties of representative species or their potential uses as food fish in Australia. Many of the species are common food fish in other countries but they are unfamiliar to the Australian industry, even though some are related to fish types caught in more temperate waters.

The CSIRO Division of Fisheries has recently completed a research programme designed to assess the current status (Young and Sainsbury, 1984) of the demersal stocks and to compare the results with those obtained on previous surveys. Officers of the Tasmanian Food Research Unit (TFRU) of the CSIRO Division of Food Research have been present on two research cruises to obtain fish of known history for investigation into their keeping qualities.

Four species representative of types obtainable and of likely commercial value were selected. This paper describes the results of sensory evaluation of the fish stored at ambient temperature and in ice and the results of taste panel evaluation of the iced stored fish.

2. MATERIALS AND METHODS

The fish were caught with a Frank and Bryce trawl out at depths of 50-80 m by the FRV SOELA on cruise SO 5/83 during November 1983 (Table 1).

The experimental outline broadly followed the guidelines listed by Lima de Santos, James and Teutcher (1981) insofar as relevant species were chosen, they were well iced, sampled regularly, assessed for sensory freshness, sampled for pH, bacteria and nucleotides (not reported here) and the sensory properties of the steamed fillets evaluated by taste panel. In addition, fish that had been frozen on board soon after catching and fish that had been stored on ice for two different periods were deep fried in butter and their acceptability assessed by taste panel.

Table 1

List of species including date of catch and subsequent treatment

Latin Name	Common name	Date Caught	Location	Shot No.	Experiment
<i>Argyrops spinifer</i>	Long spined seabream	7/11	118°49', 19°31'	88	Ambient spoils
		15/11	118°01', 19°02'	152	Iced storage/frozen
<i>Lutjanus vittatus</i>	A snapper like lutjanid	4/11	117°55', 20°40'	81	Ambient spoils
		9/11	117°43', 19°53'	107	Frozen
		16/11	117°43', 19°57'	162	Iced storage
<i>Nemipterus peronii</i>	Threadfin bream	10/11	117°45', 19°52'	114	Ambient spoils
		10/11	117°46', 19°56'	116	Frozen
		16/11	117°44', 19°56'	160	Iced storage
<i>Plectorhynchus pictus</i>	Painted sweetlip	9/11	117°43', 19°53'	107	Ambient spoils
		16/11	117°45', 19°55'	159	Iced storage/frozen
		16/11	117°43', 19°56'	161	Frozen

2.1 Ambient Spoilage

Representative fish from the four species were gutted and stored in plastic bags (to prevent dehydration) in the fish hold of the boat at ambient temperature of 25-26°C. They were assessed and sampled at convenient regular intervals.

2.2 Frozen Fish

After sorting, fish from each species were iced and allowed to pass through rigor mortis and were blast frozen the following day to a temperature near -35°C. These samples (frozen on board) were air-freighted frozen to TFRU in Hobart.

2.3 Iced Fish

Fish from the last hauls before docking were gutted and packed in crushed freshwater ice on board the boat. They were reiced when the boat docked and air-freighted to TFRU in Hobart where they were kept in ice in a cool room set at 0-1°C. The iced fish were progressively assessed and sampled for evaluation by the profile taste panel. Samples were frozen during the period of storage for later assessment of their acceptability by taste panel.

2.4 Sensory Assessment

The sensory assessment of the whole fish was done using score sheets originally developed at TFRU for the assessment of gemfish (Thresher *et al.*, 1982). These score sheets (Table 2) were simplified and weighted to provide accumulated increases in demerit points as the fish changed in odour and appearance during storage. Recently, the technique has been computerized for use in personal or hand held pocket computers (Brench and Veil, 1984). Three fish were assessed at each sampling time.

2.5 Profile panel

A round table profile panel of 8 people was used to score the odour and flavour characteristics of the cooked fish. Fillets of the fish were cooked for 15 min in polythene bags suspended in water heated to 84°C. The fillets were removed from the bags, broken into large chunks with a fork, mixed and served in a heated bowl.

Table 2

Sensory assessment score sheet

FISH IDENT.		
APPEARANCE		(V. Bright/Bright/Sl. Dull/Dull) 0 1 2 3
SKIN		(Firm/Soft) 0 1 -
SCALES		(Firm/Sl. Loose/Loose) 0 1 2
SLIME		(Absent/Sl. Slimy/Slimy/V. Slimy) 0 1 2 3
STIFFNESS		(Pre-Rigor/Rigor/Post-Rigor) 0 1 2
EYES	Clarity	(Clear/Sl. Cloudy/Cloudy) 0 1 2
	Shape	(Normal/Sl. Sunken/Sunken) 0 1 2
	Iris	(Visible/Not Visible) 0 1
	Blood	(No Blood/Sl. Bloody/V. Bloody) 0 1 2
GILLS	Colour	Characteristic (Sl. Dark) (V. Dark) (Sl. Faded) (V. Faded) 0 1 2
	Mucus	(Absent/Moderate/Excessive) 0 1 2
	Smell	(Fresh Oily) Fishy/Stale/Spoilt (Metallic, Seaweed) 1 2 3
BELLY	Discoloration	(Absent/Detectable/Moderate/Excessive) 0 1 2 3
	Firmness	(Firm/Soft/Burst) 0 1 2
VENT	Condition	Normal (Sl. Break) (Excessive) (Exudes) (Opening) 0 1 2
	Smell	(Fresh/Neutral/Fishy/Spoilt) 0 1 2 3
BELLY CAVITY	Stains	(Opalescent/Greyish/Yellow-Brown) 0 1 2
	Blood	(Red/Dark Red/Brown) 0 1 2

Profile score sheets were similar to those used previously for blue grenadier (Stetham and Bremner, 1983) and gemfish (Quaraby, Bremner and Thrower, 1982) with the exception that an unstructured 0-9 scoring scale was used (with 0 = absent, 9 = strong) rather than the previous 5-point structured scale. It was mandatory for panelists to score odour, off odour, flavour and off flavour. It was also mandatory for them to score for odour acceptability, flavour acceptability and overall acceptability on the 7-point Smiley scale. In addition, the panelists were instructed to score for the textural characteristics (Table 3) of wetness, firmness, springiness when the samples were first bitten into and also, after several chews, the characteristics of toughness, succulence and fibrousness (Howgate, 1977). Scoring for the other profile attributes was by free choice. Discussion sessions were held after each sample had been assessed.

Table 3

Textural profile terms and score range

<u>CHARACTERISTICS AFTER 1 OR 2 BITES</u>										
<u>Wetness</u>	1	2	3	4	5	6	7	8	9	
Very wet										Very dry
<u>Firmness</u>	1	2	3	4	5	6	7	8	9	
Very soft										Very firm
<u>Springiness</u>	1	2	3	4	5	6	7	8	9	
Not springy										Very springy
<u>Comments:-</u>										
<u>CHARACTERISTICS AFTER CHEWING</u>										
<u>Toughness</u>	1	2	3	4	5	6	7	8	9	
Very tender										Very tough
<u>Succulence</u>	1	2	3	4	5	6	7	8	9	
Reduces water in mouth										Increases water in mouth
<u>Fibrousness</u>	1	2	3	4	5	6	7	8	9	
Not fibrous										Very definite fibres

2.6 Acceptability Panel

2.6.1 Single presentation

Filletts of each species from fish frozen on board were presented, deep fried in bread-crumbs, to a taste panel of 22 persons who scored for flavour, texture and overall acceptability on the 7-point Smiley scale. Each species was presented singly on successive days to provide a measure of inherent acceptability unaffected by presentation of other samples. Fried potato chips were served as an accompaniment and panelists were allowed to use salt, vinegar or tomato sauce as condiments if they wished.

2.6.2 Presentation of stored samples

Deep-fried filletts from fish frozen on board and from fish stored for two different periods on ice were presented to the panel who scored them for flavour, texture and overall acceptability on the 7-point Smiley scale. The three samples for each species were presented on the same day with subsequent species presented on the following days. Fried potato chips were offered as rewards at the completion of the taste session.

2.6.3 Statistics

The acceptability taste panel results and the mandatory profile results were subjected to analysis of variance using the Genstat package.

3. RESULTS

3.1 Sensory Assessment

Accumulation of demerit points with time of storage of the fish at ambient temperature and in ice (Fig. 1) occurred in a linear fashion. The variable (time of storage and demerit points score) were highly correlated (Table 4) and the slope of the line (i.e., rate of accumulation of demerit points) was similar for the four species at each of the storage temperatures (Table 4). There were some differences between the species in those characteristics where changes were first noted (Tables 5 and 6). There were obvious differences in the nature of the changes between those stored at ambient and those stored in ice. At ambient temperature, the most noticeable characteristic to change first was the decrease in stiffness as the fish passed through rigor (Table 5); this was followed by the development of odour or mucus in the gills.

The most obvious indicator of change for fish stored in ice was the appearance of the eyes (their clarity and shape) followed by odour development and colour changes in the gills (Table 6). Thus, these initial changes were most obvious in the parts of the fish not normally eaten. It is assumed that concomitant changes are occurring in the edible flesh. This assumption is inherent in all non destructive scoring schemes which evaluate external characteristics.

The relative rate of increase in demerit points score at 25-26°C was over 40 times that at 0°C (Table 7). At temperatures above 20°C bacterial spoilage is not well defined and the growth characteristics and metabolic activities of the relevant organisms have yet to be determined (Pooni and Maad, 1984).

Table 4

Relationship between time and demerit points for fish stored at ambient and in ice

Species	Ambient (25°-26°C)			Ice		
	Y intercept	slope	correlation coefficient	Y intercept	slope	correlation coefficient
<i>A. spinifer</i>	0.2	1.29	0.99	3.8	0.76	0.94
<i>L. vittus</i>	2.2	1.14	0.88	3.3	0.71	0.96
<i>N. peronii</i>	-0.2	1.51	0.99	4.2	0.85	0.95
<i>P. pictus</i>	0.4	1.44	0.96	5.4	0.69	0.98

3.2 Profile panel

The changes that occurred in scores given by the profile taste panel for those attributes for which scoring was mandatory are shown in Figures 2 and 3. For all species odour and flavour intensity were rated lower with increasing time of storage (Fig. 2) with concomitant higher scores for off odour and off flavour. Similarly, the scores for odour, flavour and overall acceptabilities decreased with time of storage (Fig. 3).

The main changes are summarized as follows:

Odour intensity

L. vittus had a significantly higher odour intensity ($P < 0.05$) than the other species. During storage odour intensity decreased significantly in all species ($P < 0.001$); *P. pictus* showed the greatest decrease.

Table 5

Attributes contributing to score change for fish held at ambient temperature

Score	<i>A. spinifer</i>	Attributes involved in score change			
		<i>L. vittus</i>	<i>H. peronii</i>	<i>P. pictus</i>	
<10	Scales; Stiffness; Eyes-shape, iris; Gills-colour, mucus	Stiffness; Eyes-clarity, shape, iris, blood; Gills-colour	Appearance; skin Scales; Stiffness; Eyes-clarity, shape; Gills-colour, smell; Belly cavity-blood	Appearance; Stiffness; Eyes-clarity, shape, iris; Gills-colour	
<15	Appearance; Scales Eyes-clarity, blood; Gills-colour; Belly cavity-stains, blood	Scales; Eyes-clarity, blood; Gills-smell	Eyes-clarity, shape Gills mucous. Belly cavity-stains	Scales; Eyes-clarity, shape, blood; Gills-mucous; Belly cavity-blood	
<19	Appearance; Skin; Eyes-clarity; Belly cavity-stains, blood	Appearance; Skin; Eyes-shape, iris; Belly cavity-stains, blood	Appearance; Scales; eyes-shape; Gills colour, smell Belly cavity-stains, blood	Appearance; Skin; Gills-colour, smell; Belly cavity-stains, blood	

Table 6

Attributes contributing to total score for fish held in ice

Score	<i>A. spinifer</i>	Attributes involved in score change			
		<i>L. vittus</i>	<i>H. peronii</i>	<i>P. pictus</i>	
<6	Stiffness; Eyes-shape; Gills-colour, mucus; Belly cavity-blood	Appearance; Slime; Stiffness; Gills-colour, smell	Stiffness; Gills-colour, smell; Belly cavity-blood	Appearance; slime; Stiffness; Gills-colour, mucus	
<13	Appearance; Skin; Scales; Eyes-clarity; Gills-colour, mucus, smell	Appearance; Stiffness; Eyes-clarity; Gills-colour, mucus smell; Belly cavity-stains, blood	Appearance; Skin; Eyes-colour, Shape, iris; Gills-smell	Appearance; Skin; Stiffness; Eyes-shape, iris; Gills-colour, smell; Belly cavity-blood	

Table 7

Spoilage rates in ice and at ambient temperature

	In ice (Demerit points/hour)	At ambient (Demerit points/hour)	Rate relative to 0°C
<i>A. spinifer</i>	0.032	1.29	40
<i>L. vittus</i>	0.030	1.14	38
<i>N. peronii</i>	0.035	1.51	43
<i>P. pictus</i>	0.029	1.44	50

Off odour

There were negligible scores given to off odour at the first taste. All species increased in off odour rating ($P < 0.001$) with *N. peronii* showing the least increase.

Flavour intensity

Initially, *L. vittus*, *N. peronii* and *A. spinifer* had similar ratings for odour intensity but *P. pictus* was rated significantly lower ($P < 0.05$) than the first two. The flavour intensity of all species decreased ($P < 0.001$) but *N. peronii* showed the least decrease.

Off flavour

There were negligible ratings given to off flavour at the first taste and all species showed significant increases in off flavour during storage ($P < 0.001$). *P. pictus* was rated as having significantly ($P < 0.05$) more off flavour than the other three species.

Odour acceptability

Initially all species were equally acceptable in odour. They all decreased in acceptability during storage ($P < 0.001$) with *P. pictus* showing the greatest decrease.

Flavour acceptability

All four species were equally acceptable in flavour at the first taste, and all declined in acceptability during storage ($P < 0.01$). *P. pictus* became least acceptable.

Overall acceptability

Initially, *A. spinifer*, *N. peronii* and *L. vittus* were equally acceptable with *P. pictus* of significantly lower ($P < 0.05$) acceptability than *A. spinifer* or *N. peronii*. All species decreased in acceptability with time of storage.

Texture profile

The results for the textural profiles of the fish at the first taste are listed in Table 8. *P. pictus* was rated much softer in texture than the other species with significantly lower scores ($P < 0.001$) for firmness, springiness, toughness, succulence and fibrousness. The only fish to alter in textural properties was *N. peronii* which with time of storage was progressively rated higher in firmness, springiness, toughness and fibrousness scores, although none of changes in score quite reached statistical significance. Such changes in iced storage are unusual; it is generally considered that fish flesh softens due to enzymatic activity and (eventually) microbial activity when stored in ice.

3.3 Profile Attributes

The profile panel results obtained for odour and flavour at the first taste have been plotted as bar graph profiles (Fig. 4). Only those attributes which were scored by at least two panelists are shown. For display purposes the scale used is relative but proportional to the total panel score. There are obvious similarities and differences between the species. The major changes in the odour and flavour profiles that occurred during storage of the fish in ice are listed in Table 9.

Table 8

Textural profiles of fish at first tests

Attribute	<i>A. spinifer</i>	<i>L. vittus</i>	<i>N. peronii</i>	<i>P. pictus</i>
Wetness	4.1	4.4	4.6	4.6
Firmness	4.6	3.6	4.1	2.3 ^{a/}
Springiness	4.0	5.0	4.0	2.6 ^{a/}
Toughness	4.0	3.6	3.9	2.8 ^{a/}
Succulence	4.6	4.3	5.3	3.6 ^{a/}
Fibrousness	5.0	4.0	4.1	2.0 ^{a/}

^{a/} Significantly different ($P < 0.001$) from the scores for the other three species

Table 9

Changes in profile descriptor terms for fish stored in ice

Changes	<i>A. spinifer</i>	<i>L. vittus</i>	<i>N. peronii</i>	<i>P. pictus</i>
Odour terms lost on storage	chicken	<u>seaweedy</u> chicken <u>roast meat</u>	<u>seaweedy</u> boiled clothes	<u>seaweedy</u> sweet chicken <u>roast meat</u>
Odour terms gained on storage	<u>sour cloths</u> acid	<u>wet straw</u> rubbery rancid oily sour cloths	<u>burnt</u> musty acid <u>sour cloths</u>	<u>rubbery</u> <u>acid</u>
Flavour terms lost on storage	<u>sweet</u> <u>salty</u> <u>chicken</u>	<u>sweet</u> salty creamy	<u>sweet</u> <u>salty</u> chicken creamy	<u>sweet</u> <u>salty</u> chicken creamy
Flavour terms gained on storage	<u>soapy</u> <u>musty</u>	<u>burnt</u> <u>rancid oily</u>	<u>metallic</u> <u>sour</u> oatmeal blood	<u>astringent</u> rancid oily liver

- major changes underlined

After storage, lower scores were given to those attributes which could be considered desirable, such as seaweedy, chicken and sweet for odour, and sweet, salty or creamy for flavour. Other attributes which could be considered as less desirable such as sour cloths or rubbery for odour, and musty or rancid oily for flavour, were scored for the stored fish. The pattern of change in odour and flavour profile was slightly different for each species (Table 9).

3.4 Acceptability Panel

3.4.1. Single presentation

Higher scores were given by the panel to *A. spinifer* and *L. vittus* for acceptability than were given to the other two species (Table 10). All the scores were relatively high and the samples thus compared favourably with results obtained from similar panels for high-priced species such as scallops and *travalla* (CSIRO, 1984).

Table 10

Acceptability of deep fried NSW fish using single presentation

Acceptability	<i>A. spinifer</i>	<i>L. vittus</i>	<i>N. peronii</i>	<i>P. pictus</i>
Flavour	6.0 ^{ab}	6.2 ^a	4.9 ^b	5.5 ^c
Texture	6.1	6.1	5.4	6.0
Overall	6.0 ^a	6.1 ^a	5.1 ^c	5.6 ^b

^a/_b/ Significantly different ($P < 0.05$)

^c/ Significantly different ($P < 0.01$)

3.4.2. Stored sample presentation

The pooled scores across all four species for flavour and overall acceptability for these samples frozen on board were significantly higher ($P < 0.05$) than for those fish that had been stored in ice (Table 11). The acceptabilities of all four species stored in ice remained high.

Table 11

Acceptability of deep fried fish from NW shelf

Acceptability	Sample	<i>A. spinifer</i>	<i>L. vittus</i>	<i>N. peronii</i>	<i>P. pictus</i>
Flavour	Frozen on board ^a /	5.8	5.7	6.1	5.3
	intermediate sample ^b /	5.5	5.3	5.4	4.8
	final sample	5.3	5.3	5.1	5.3
Texture	Frozen on board	5.7	5.6	6.0	5.1
	intermediate sample	5.4	5.2	5.5	4.9
	final sample	5.2	5.6	5.3	5.4
Overall	frozen on board ^a /	5.7	5.6	6.0	5.1
	intermediate	5.4	5.2	5.5	4.9
	final sample	5.2	5.6	5.3	5.4

^a/ Fish from frozen on board treatment rated significantly ($P < 0.05$) higher in acceptability

^b/ *A. spinifer* frozen after 13 days and 24 days on ice

L. vittus frozen after 12 and 16 days on ice

N. peronii and *P. pictus* frozen after 12 and 23 days on ice

The inherent textural softness of *P. pictus* and the toughening on storage of *N. peronii* detected by the profile panel which tasted steamed fillets was no longer noticeable when the fish was deep fried. The deteriorative changes noted in the profile characteristics (Table 9; Figs. 3, 4 and 5) were not sufficiently relevant to downgrade the fish when served in this way.

Samplings of *L. vittus* were frozen for this acceptability trial after 16 days storage in ice because of some adverse comments given by the profile panel, but it appears the decision to freeze them may have been taken prematurely. There was insufficient material to freeze other samples after either 20 or 23 days storage.

4. DISCUSSION AND CONCLUSIONS

The four tropical species of fish investigated (*A. spinifer*, *L. vittus*, *N. peronii* and *P. pictus*) possess characteristics that make them highly acceptable fish and freezing on board produces an excellent product. During iced storage for up to 23 days obvious changes occurred in all four species as indicated by sensory evaluation of the raw whole fish and taste panel profiles on the cooked fish. Nevertheless, these changes only marginally decreased the acceptability ratings when fillets were presented deep fried. This agrees with the results of Lalett and Bremner (1979) who concluded that taste panel work to determine the acceptability of fish fingers should be done on the fish fingers themselves not the minces they were made from. Deep frying in batter or bread-crumbs is a popular way of serving fish in Australia but it is likely that by using other methods of preparation e.g., baked whole fish, panelists would have given the fish lower ratings for acceptability. For the fishery to develop as one which lands fresh fish for the domestic market, and for it to be economically viable, it is likely that high prices will need to be paid for the catch. The results of these experiments indicate that very little detectable change occurs in any of the species up to 10 days in ice after catching, and at this stage the fish is thus suitable for the top bracket of the catering trade.

5. REFERENCES

- Branch, A.C. and A.M.A. Vail, Bringing fish inspection into the computer age. Food Technol., Aust., 1984 36
- CSIRO Division of Food Research, Annual report 1983-84. Canberra, CSIRO, Division of Food Research 1984
- Bowgate, P., Aspects of fish texture. In Sensory properties of foods, edited by G.G. Birch, 1977 J.G. Brennan and K.J. Parker. London, Applied Science Publishers Ltd., pp. 249-69
- Lalett, G.M. and N.A. Bremner, Evaluating acceptability of fish minces and fish fingers from sensory variables. J. Food Technol., 14:389-404
- Lima dos Santos, C.A.M., D. James and P. Tautacher, Guidelines for chilled storage experiments. 1981 FAO Fish. Tech. Pap., (210):22 p.
- Pooni, G.S. and G.C. Mead, Prospective use of temperature function integration for predicting the shelf life of non-frozen poultry-meat products. Food Microbiol., 1:67-78
- Quarby, A.R., M.A. Bremner and S.J. Thrower, On board handling of gemfish. 2. Sensory profiles. 1982 Aust. Fish., 41(11):42-5
- Sainsbury, K.J., CSIRO defining fish stocks on NW shelf. Aust. Fish., 38(3):4-12 1979
- Stetham, J.A. and N.A. Bremner, Effect of potassium sorbate on spoilage of grenadier (*Macrurus novaezealandiae*) as assessed by microbiology and sensory profiles. J. Food Prot., 46: 1084-91
- Thrower, S.J., et al., On board handling of gemfish. 1. Importance of chilling and gutting. Aust. Fish., 41(11):38-41 1982
- Young, P.C. and K.J. Sainsbury, CSIRO's North West Shelf Program. Aust. Fish., 43 1984

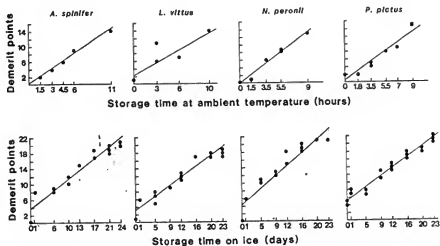


Figure 1 Change in demerit point score with time of storage at ambient temperature and in ice

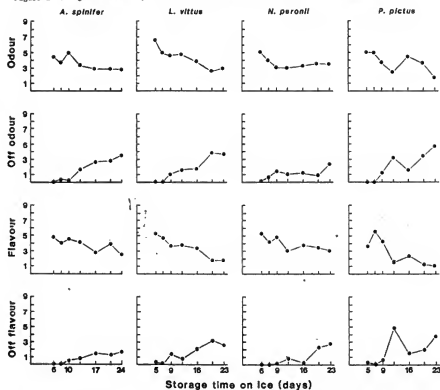


Figure 2 Changes in odour, off odour, flavour and off flavour ratings with time of storage in ice

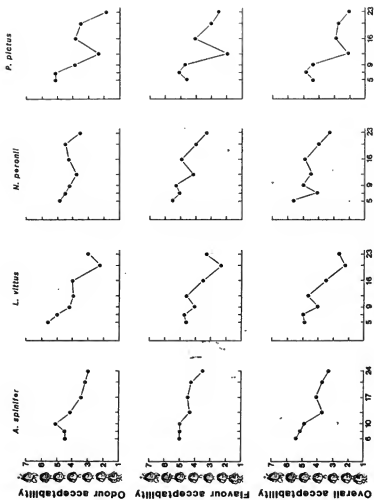


Figure 3 Changes in odour, flavour and overall acceptability with time of storage in ice

NWS SPECIES FLAVOUR PROFILE - FIRST TASTING

DESCRIPTOR	<i>A.spinifer</i>	<i>L.vittus</i>	<i>N.peronii</i>	<i>P.pictus</i>
Sweet				
Salty				
Creasy				
Buttery				
Cheesey				
Fresh Oily				
Waxy				
Soapy				
Boiled Potatoes				
Boiled Meat				
Roast Meat				
Chicken				
Blood				
Liver				
Cardboard				

NWS SPECIES ODOUR PROFILE - FIRST TASTING

DESCRIPTOR	<i>A.spinifer</i>	<i>L.vittus</i>	<i>N.peronii</i>	<i>P.pictus</i>
Seaweedy				
Shellfish				
Cheesey				
Boiled Milk				
Sweet				
Roast Meat				
Chicken				
Boiled Potatoes				
Boiled Clothes				
Lemon				

Figure 4 Odour and flavour profiles at first taste session

STORAGE LIFE OF RABBIT FISH (*Siganus* sp.)
DURING ICING

by

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ABSTRACT

Rabbit fish is one of the increasingly important species for mariculture in Indonesia. It has a tender and delicious meat with relatively few bones. Therefore, it has a good demand for both export and local consumption. Studies on the storage life of rabbit fish have been carried out to determine shelf life as well as quality changes during ice storage. The results showed that at ambient temperature rabbit fish can be stored up to 12 h or 13 days in chilled storage. The acceptability of chilled stored fish can be extended up to 17 days if the fish were cooked. Results on biochemical, microbiological and sensory changes are presented and discussed.

1. INTRODUCTION

Rabbit fish (*Siganus* sp.) plays an increasingly important role among economically valued species. The popularity of the fish was originally confined to people in South Sulawesi and its vicinity. In recent years, however, rabbit fish is being experimentally cultivated in many areas which was pioneered by a collaborative research project between JICA and the Marine Fisheries Research Station, Serang.

The present studies are intended to determine the shelf life of rabbit fish during iced and ambient temperature storages. Since fresh rabbit fish commands a high price proper handling practices can extend its shelf life and maintain freshness during transport and marketing in wider areas.

2. MATERIALS AND METHODS

Studies on quality deterioration of rabbit fish at ambient temperature were carried out at the Marine Fisheries Research Station, Serang, whereas iced storage was conducted at the Research Institute for Fish Technology, Jakarta.

2.1 Raw Material

Rabbit fish used in this study was obtained from Mariculture Research Station, Serang (West Java). The samples weighed an average of 300 g and were approximately 9 months old.

Immediately after catching the fish were divided into 2 lots. The first lot was kept in a plastic tray at ambient temperature, while the second lot was packed in a 20-kg capacity insulated box with crushed ice.

2.2 Quality Assessment

Quality assessment of uniced and iced rabbit fish was performed at 3 h and 2 days intervals respectively. Sensory analysis used the descriptive method using a hedonic scale of 1-5 for raw and 1-10 for cooked fish. Chemical analysis included total volatile bases (TVB), trimethylamine (TMA) and pH determination. Microbiological analysis included total plate counts on nutrient agar; H_2S producers counts on iron agar and *Enterobacteriaceae* count on VRBGA. All plates were incubated at 30° and 10°C.

In addition, temperature profile of the fish storage room and relative humidity were monitored.

3. RESULTS

3.1 Physical Properties of the Fish

The physical properties of the rabbit fish used in this study are presented in Table 1. Temperature and relative humidity fluctuations of storage room are shown in Figure 1 and the

temperature of iced stored fish in Table 2. Sensory evaluation results are given in Figures 2 and 3 which are based on the descriptive scorecard outlined in Tables 3 and 4.

Table 1

Physical properties of rabbit fish

Number	Weight (g)	Total length (cm)	Standard length (cm)	Thickness (cm)
1	312	24.0	20.5	2.85
2	320	22.5	19.5	2.75
3	240	21.5	19.5	2.65
4	358	24.0	21.0	3.10
5	290	23.5	20.5	2.80
6	293	24.0	20.5	2.75
7	310	23.5	19.5	2.60
8	215	22.0	19.5	2.35
9	300	24.0	20.5	2.50
10	384	25.5	22.5	2.95
Total	3,022	234.5	203.5	27.30
X	302.2	23.45	20.35	2.73

Table 2

Temperature (°C) fluctuation of the fish stored in ice

Days	0	2	4	6	8	10	12	14	16	18	20
Fish I	28.8	0.2	-	0.4	0.2	0.2	0.2	0.1	0.0	0.1	0.2
II	28.8	0.2	-	0.4	0.2	0.2	0.1	0.0	0.0	0.1	0.2
Media I	0.1	0.1	-	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.1
II		0.1	-	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0

4. DISCUSSION

4.1 Ambient Storage

When the experiment was started, the ambient temperature was 32°C whereas the temperature of the fish was 28.8°C. The ambient temperature increased slightly at 4.00 p.m. but gradually declined at a very slow rate. Minimum temperature (28°C) was recorded at 6.00 p.m. which corresponds to the temperature of the fish about 26.5°C.

Thermohydrograph data showed that there was correlation between the ambient temperature and relative humidity (RH), since the temperature increase would increase the RH. It was noted that when the ambient temperature was 32°C, the RH was 70% and when the temperature dropped to 28°C the RH was 96% (Figure 1).

4.2 Chilled Storage

The results showed that the temperature at the centre of the fish muscle kept in crushed ice was close to the temperature of the medium. The temperature of the fish varied between 0° to 4°C whereas the temperature of the chilling medium was between 0° to 0.2°C (Table 2).

4.3 Sensory Changes

4.3.1 Room temperature storage

Rabbit fish kept at ambient temperature were rejected by the taste panel after 12 h. Shelf life of rabbit fish was longer than *Puntius* or *Sepat siam* with only 7 h (Utomo *et al.*, 1984). The raw odours and general appearance of the gills deteriorated rapidly. Based on textural quality, rabbit fish can be stored for 18 h because of its hard and compact skin. Cooking slightly improved the quality and shelf life was extended by one day.

4.4 Chilled Storage

Chilling rabbit fish extended the shelf life up to 13 days. This was longer than the fresh-water *Puntius* with 11 days and *Sepat siam* with 9 days (Utomo *et al.*, 1984); but shorter than *V. seahii* and *L. subviridis* with 21 and 22 days respectively (Anon., 1984). In contrast with ambient storage, chilled storage maintained the odour and textural properties of the fish much longer than other sensory parameters. The eyes and muscle section rapidly deteriorated, but after prolonged storage fish were rejected on account of the condition of the gills and odour. However, sensory evaluation of cooked fish indicated that the fish could be stored in ice for up to 19 days. This fact further indicated that cooking could improve the organoleptic properties of the fish.

4.5 Chemical Analysis

Proximate analysis showed that rabbit fish contained 73.9% water, 19.53% protein, 4.32% fat, 1.67% ash and 0.58% salt.

As shown in Figures 4 and 5 the TVB contents tended to increase under both storage conditions as spoilage advanced, although they showed different patterns. The TVB content of iced stored rabbit fish increased relatively slowly initially but rapidly as the quality of the fish approached the border line of acceptance and there was no significant increase at the end of storage. On the other hand, rabbit fish stored at ambient temperature showed a rapid increase in TVB values from the third hour of storage and steadily increased until the fish was rejected by panelists after 12 h. The initial TVB content was 9.27 mg% when rejected by panelists after 12-h storage was 19.99 mg% and further increased to 75.03 mg% after 18-h storage. Rabbit fish stored at chilled temperature had a TVB content of 13.33 mg% when approaching the border line of acceptance (13 days) and slowly increased to 19.32 and 24.20 mg% when rejected by panelists (14 days). These values are lower than TVB content of iced skipjack when rejected by panelists (25.30-27.06 mg%) (Saleh, Abdul Sari and Nur Retnowati, 1984).

4.6 Microbiological Analysis

As shown in Figures 6 and 7 the total plate count (TPC), H_2S producers and *Enterobacteriaceae* tend to increase during ambient temperature or chilled storage although there are slight fluctuations for TPC, H_2S producers and *Enterobacteriaceae* numbers on chilled storage. There was evidence that rabbit fish subjected to room temperature storage carry more mesophilic bacteria than psychrophiles. On the contrary, they had more psychrophilic H_2S producers and equal number of mesophilic and psychrophilic *Enterobacteriaceae*. Rabbit fish stored under chilled temperature also showed a higher total mesophilic count compared to psychrophilic bacteria. Unexpectedly, the number of mesophilic coliforms and H_2S producers were higher than the psychrophiles. Evidently the mesophiles which originated from the fish were able to withstand or adapt and multiply under chilled storage. Rabbit fish carried smaller amounts of psychrophiles, which may account for the effectiveness of icing in extending the shelf life of the fish.

5. CONCLUSION

Rabbit fish can be stored up to 12 h at ambient temperature and 13 days chilled. This shelf life is longer than freshwater *Puntius* or *Sepat siam* but shorter than mullets (*V. seahii* or *L. subviridis*). Rabbit fish also appear to carry smaller numbers of psychrophiles and more mesophiles, which may account for the effectiveness of chilling in extending the shelf life.

6. REFERENCES

- Saleh, M., Abdul Sari and Nur Retnowati, Effects of icing on the quality of canned skipjack 1984 (*Katsuwonus pelamis*). Research Report for Fishery Technology, 26 RIFT, Jakarta
- Utomo, B.S.B., T. Sofyan Ilyas, Dwi Suryaningrum and Umi Rahayu, Storage of freshwater fish in 1984 crushed ice. Research Report for Fishery Technology, 26, RIFT, Jakarta
- Anon., Progress Report ACIAR Project on prediction and control of fish spoilage. Unpublished 1984

Table 3

Changes in descriptive sensory evaluation of
rabbit fish stored at ambient temperature

Hours	Eyes	Gills	Skin	Odour	Texture
0	Opaque, shiny with clear margins	Bright red, transparent thin slime, gill filaments tight, with specific fishy odour	Slime very thin and transparent, shiny with green yellowish, shiny, tiny spots	Fresh, fishy odour - resembles seaweed odour	Compact, elastic flesh, whitish. Intact belly walls and visceral organs
3	Slightly dull	There is barely describable odour detected	Slime slightly sticky	-do-	-do-
6	Eyes less opaque, slightly dull, greyish with unclear margins	Dark red slime, slightly thick, Fishy odour	Dull appearance, dark yellowish spots becoming blurred, slime slightly sticky	Seaweed odour starts to disappear, fishy odour more pronounced with slight metallic odour	Fish flesh slightly pale, connective tissues with clear margins
9	Flat eyes, dull greyish, with unclear margins	dark brown, thick slime, off odour	Slightly thick and sticky slime, dull appearance and less specific colour	Fishy odour with slightly prominent acid metallic odour	Compact but less elastic. Fish flesh pale, connective tissues slightly loose, soft and moist
12	-do-	Off odour, spoiled	Thick and sticky slime, dull, original colour disappeared, off odour	Off odour	Less elastic, fish flesh dull, soft, loose and moist
15	Eyes sunken, dull greyish and slimy	Dark brownish, thick slime, off odour	Slime red brownish, thick and sticky, original colour disappeared	Metallic odour and strong off odour	Soft, finger impressions retained, flesh dull, loose and moist
18	Swollen, dark grey and slimy	Dark brownish, thick and sticky slime, strong off odour	Belly swollen, brownish slime, sticky and dull off odour	Spoiled	Very soft, disintegrated. Dull flesh, loose and watery. Belly disintegrated

Table 4

Descriptive sensory evaluation on the quality changes of rabbit fish during chill storage

Days	Eyes	Gills	Skin	Odour	Texture
0	Opaque, clear dark shiny with clear margins	Bright red, thin, transparent slime, tight filaments, fresh characteristic fishy odour	Thin, transparent skin, shiny. With green yellowish spots, tiny and with scales	Fresh fishy odour, seaweedy	Firm, compact, elastic flesh. Whitish, transparent. Belly wall and visceral organs are intact
2	Slightly flat, dark shiny, clear margins	Red brownish, thin slime, fresh fishy odour, tight filaments	Thin transparent slime, fresh fishy odour, bright, compact and elastic	-do-	-do-
4	Slightly sunken, dark, clear, shiny, greenish	-do-	-do-	-do-	Solid, compact, elastic. Fish flesh transparent but slightly soft
6	Sunken eyes, dark transparent but slightly blurred, no distinct margins	Red brownish, pale, slime rather thick with slightly metallic odour	-do-	Slightly neutral with the additional strange odour	-do-
8	Eyes become dull	Red brownish, pale slime; metallic fishy odour	Thin slime, fairly elastic, slightly dull, almost neutral odour	Neutral, slightly fishy and metallic odour	Compact and elastic, fish flesh transparent, slightly soft and loose
10	Sunken eyes, dark reddish, dull and greyish	Pale brownish, thick slime, fishy metallic acid odour	Thick slime and dull	Slightly fishy and metallic odour	Compact but less elastic. Fish flesh white, dull, soft and loose
12	-do-	-do-	Slime slightly thick and dull with slight discoloration	Strong metallic acid odour	-do-
14	Sunken eyes, dark reddish, dull and greyish	Slight off odour	Slime slightly thick and dull, with slight discoloration	Strong metallic acid odour	Finger impressions retained. Fish flesh soft and watery
16	Sunken eyes, grayish with reddish colour	Red brownish, thick slime and off odour	-do-	Very fishy, strong metallic acid odour	Fish flesh soft and loose. Meat layers easily separated, bones protruding
18	Swollen eyes, dull, reddish	Dark brown, thick slime, off odour and dull	Thin slime slightly sticky, dull in appearance	Off odour	Very soft flesh, almost disintegrated, bones protruding, belly burst, off odour
20	-do-	-do-	Thick slime, sticky, dull brownish colour	-do-	-do-

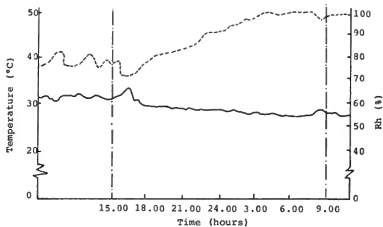


Figure 1 Temperature and relative humidity fluctuations of the storage room

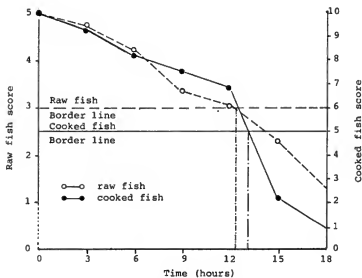


Figure 2 Organoleptic changes of rabbit fish during storage

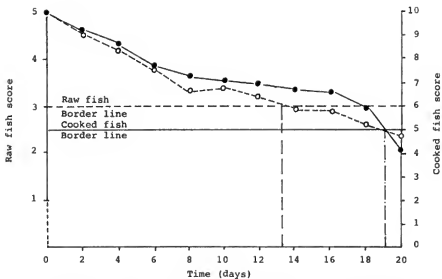


Figure 3 Organoleptic score of raw rabbit fish (*Siganus* sp.) ○—○ and cooked rabbit fish ●—● stored at chilled temperature

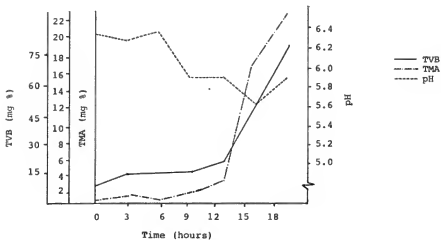


Figure 4 Chemical change of rabbit fish during room temperature storage

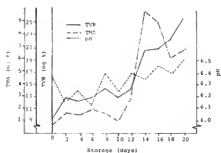


Figure 5 Chemical changes of rabbit fish during ice storage

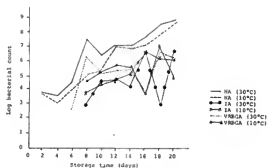


Figure 6 Microbiological changes of rabbit fish during chilled temperature storage

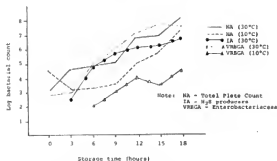


Figure 7 Microbiological changes of rabbit fish during room temperature storage

DO TROPICAL FISH KEEP LONGER IN ICE THAN TEMPERATE FISH:
THE CIRCUMSTANTIAL AND DEFINITIVE APPROACHES

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ABSTRACT

The circumstantial approach has involved harvesting mullets and tilapia from waters of different temperature, then determining shelf life in ice. *Mugil cephalus* captured in Australia had shelf lives in ice of 16-24 days, gutted and 20-26 days, ungutted. Fish from warmer (21-23°C) waters kept longer than those from colder (8-14°C) waters. In the Philippines, *Liza subviridis* and *Valamugil sebastes* kept 29 and 21-22 days, respectively. For all trials bacterial counts rose from an initial 10^3 - 10^4 /g for Australian fish, and 10^4 - 10^5 /g for Philippine fish to levels at rejection of 10^7 - 10^8 /g and 10^7 - 10^6 /g, respectively, sulphide producers comprising around 0.1-1.0% initially and 10% at rejection. K-values ranged from 16-36% in fish newly captured to 75-92% when the fish was rejected by taste panels. In five trials the storage life of tilapia (*Oreochromis niloticus*) in ice varied from 21 to 27 days. Torrymeter readings fell progressively from an initial level of 12 to 3-6 at rejection. Total bacterial count rose from 10^3 - 10^4 /g initially to 10^8 - 10^9 /g at rejection with sulphide producers comprising 1-10% of the total microflora throughout the storage period. TVN levels exceeded 30 mg TVN-N/100 g at rejection. K-values rose from early levels of 11-22% to 83-100% at rejection. The present study adds to the body of circumstantial evidence which states that fish from warm waters keep longer in ice than those from cold waters. The definitive approach has involved acclimatizing tilapia in tanks at either 15°C or 35°C prior to sacrificing and storing in ice. Fish from warm waters kept 26 days in ice, compared with 22 days for fish from cold waters.

1. INTRODUCTION

It was Disney *et al.* (1971) who first alerted fish scientists and technologists to the fact that fish from tropical waters might have longer storage life in ice than fish taken from cold waters. Later Lima dos Santos (1981) and Poulter, Curran and Disney (1981) presented storage data for a number of studies carried out by FAO and Tropical Products Institute staff which indicated that while cold/temperate species kept an average of 14 days in ice, tropical species kept 21 days. The most widely-accepted theory for the longer storage life of tropical species has been propounded by Shewan (1977) and deals with the relative proportions of psychrotrophic bacteria in waters of differing temperature; colder waters are thought to have higher numbers of psychrotrophs which, in turn, coat the fish and shorten the shelf life, compared with tropical species.

In order to enhance knowledge on the shelf lives and mechanisms of spoilage in ice of tropical, as opposed to temperate and coldwater fish species, the Fish Technology Working Party of the Indo-Pacific Fisheries Council (IPFC), in its Malaysia meeting of November 1982, set several strategies. One possible mode was to locate species which grew over a wide temperature range and a group which fulfilled this criterion was mullets, a group which has been reported from tropical waters around 30°C and cold waters (8°C) in the southern states of Australia, covering a range in latitude from 20°N to 40°S.

Another genus capable of growing over a wide temperature band is tilapia (*Oreochromis*), particularly *O. niloticus*, which has been reported as surviving extremes of 8° and 42°C (Chervinski, 1982; Philippert and Ruwet, 1982). As well, tilapia have been located in water systems at altitudes over 2 000 m in Uganda and Ecuador, the implication being that growth and reproduction have been unimpaired by the cold temperatures at high altitude (Chimits, 1957; FAO, 1977).

In the Philippines tilapia are farmed in ponds, the temperatures of which vary both seasonally and diurnally between a range of 20°-35°C. Interestingly, in the present context, it was learned

of tilapia being farmed in the mountain regions of Luzon. The opportunity presented itself, therefore, to compare tilapia from "cold" waters in Banasue and La Trinidad with those from "warm" waters of Laguna de Bay and Central Luzon. Accordingly, batches of tilapia were obtained from these sources and stored in ice. In all trials *Oreochromis niloticus* was used, the name deriving from Trewavas (1982) and replacing *Sarotherodon niloticus*.

Another strategy set at the 1982 IPFC Working Party was that of acclimatizing fish in systems of widely differing temperature, then sacrificing and storing in ice. As part of a cooperative research programme between the UPV College of Fisheries and the Royal Melbourne Institute of Technology work toward both the circumstantial and the definitive approaches to storage life in ice of fish from waters of different temperature has been carried out, funded by the Australian Centre for International Agricultural Research (CIAR). The results of this study are now reported.

Data from work submitted for publication elsewhere are included; these data have been gained from several studies in Australia and the Philippines and the aid of a number of colleagues is acknowledged.

2. MATERIALS AND METHODS

Mulletts have been captured both in Australia and in the Philippines. *Mugil cephalus* was taken by netting in the Gippeland river system near Bairnsdale, Victoria, in winter 1981, 1982 and 1983 when the water temperatures were 9°, 8° and 14°C, respectively. *M. cephalus* was also obtained near Brisbane, Queensland, in 1981 and 1983 when the sea temperatures were 23° and 21°C, respectively.

Valamugil sebalt was obtained on three occasions in late-1983, early-1984 from Manila Bay by fishermen operating from Cavite. See temperature was 28°C.

Liza subviridis was netted in Neujan Lake, Mindoro Oriental and transported to holding ponds at the SEAFDEC Station, Neujan; water temperature was 26°-28°C.

Tilapia were obtained for five storage trials. In Trial 1 fish were taken from Cordona, Laguna de Bay, in April 1983 when the lake temperature was around 28°C. In Trial 2 tilapia were harvested from a private farm based on the rice paddy system in Banasue in April 1983 (water temperature 22°C). Fish for Trial 3 also came from Banasue in December 1983, from a Bureau of Fisheries and Aquatic Resources (BFAR) farm, from waters of 16°C. Trial-4 fish also were obtained from a BFAR farm at La Trinidad in March 1984, the ponds being fed by a spring at 16°C. For Trial 5 tilapia were harvested in March 1984 from the Aquaculture Center of Central Luzon State University.

In all cases fish were quickly washed in clean water from the same water system from which they had come to remove adhering soil and then placed in an insulated chest containing ice. The ratio of ice to fish was maintained at least 1:1 throughout the storage life of the fish, water being drained from the insulated chest regularly.

Tilapia, 82 pieces, were randomly divided into two groups and stocked in twin tanks of water fed from a deep well at the Institute of Fisheries Development and Research (IFDR), University of the Philippines in the Visayas, College of Fisheries. Fish had an average length of 13.4 cm and weight 41.4 g. The temperature in the "coldwater" tank was gradually lowered over a nine-day period after stocking from 28° to 15°-17°C, while that in the "warmwater" tank was raised from 28° to 35°-37°C. After 12 days at either 15°-17°C or at 35°-37°C fish were harvested, sacrificed and iced as described previously. At appropriate periods throughout storage fish were withdrawn for sensory evaluation by a trained taste panel, and determination of Torrymeter reading, bacterial levels and K-value.

Torrymeter readings were carried out using a G.R. Torrymeter (G.R. Electronics Ltd., Scotland) in which, for each fish, readings were taken on each side in the anterior-dorsal region and the mean calculated. At each sampling several fish were subjected to Torrymeter reading.

Total volatile nitrogen was determined on a sample of flesh (25 g) which was homogenized in trichloroacetic acid (5%) and the filtrate analysed by the Conway microdiffusion technique (Conway, 1968).

K-value: Flesh (20 g) from the anterior-dorsal region was extracted in perchloric acid (0.6 M, 50 ml), filtered, and the filtrate adjusted to pH 6.4 with KOH. After overnight storage in a refrigerator, crystals of potassium perchlorate were separated and the filtrate frozen until all extracts were available at termination of the experiment and K-value could be determined using the method of Uchiyama and Uchiyama (1970).

Bacterial counts were carried out by macerating flesh (10-20 g) in a decimal dilution of peptone water (0.1% w.v.) in either a Colworth Stomacher for 30 sec or in a Bmix macerator for 30 sec. Serial dilutions were prepared in peptone water (0.1%) which were plated on to a peptone iron agar (Jensen and Schultz, 1980) and incubated at 10°C for five-seven days when colonies were counted. Black colonies were enumerated separately from non-black colonies, both components on the plate going to make the total plate count.

Sensory analysis: A trained taste panel evaluated fish both in the raw and cooked state. At rejection raw tilapia had loose scales beneath a thick layer of yellowish slime; the belly was soft and, in some cases, had burst; the eyes were sunken and the gills had a strong fishy odour. For taste panel evaluation filets were removed and trimmed to remove gut cavity, wrapped in aluminium foil and steamed for 10 min. At rejection fish had a texture which was soft and mushy, an odour which was ammoniacal and putrid, and a bitter flavour. On the basis of both raw and cooked evaluation the storage life in ice was determined.

3. RESULTS AND DISCUSSION

For *Mugil cephalus* shelf lives ranged from 16 to 24 days for gutted and from 20 to 26 days for ungutted fish (Table 1). Fish used in these studies were fatty and the prime reason for taste panel rejection was a rancid flavour accompanied by a softening of texture. Fish from the two "warmwater" trials kept longer than those from the three "coldwater" trials.

By contrast, the two Philippine species examined, *Liza subviridis* and *Valamugil seheli* were low in fat (2-4%) and rejection of both these species was due to bitterness plus ammoniacal odours. *L. subviridis* had a storage life in ice of 28-29 days, while *V. seheli* kept 21-22 days in ice.

Among the objective tests, Torrymeter readings fell progressively within the relatively narrow range of 10, on catching to 3-4, at rejection. Bacterial levels rose in *M. cephalus* from an initial 10^3 - 10^4 to 10^7 - 10^8 /g at rejection, with black colonies, less than 10^2 /g (not detected on 10^2 dilution plates) initially, rose to 10^5 - 10^8 /g at rejection, and comprised 1-10% of the total microflora. For the two Philippine species initial bacterial counts were rather higher than on Australian fish, 10^4 - 10^5 /g; Cann (1977) has commented on the higher counts obtained on tropical, compared with temperate or coldwater fish, immediately on capture. Rejection levels of 10^7 - 10^8 /g were typical for *L. subviridis* and *V. seheli*. Black colonies rose from 10^2 /g to 10^7 /g at rejection, and comprised at least 10% of the total microflora (Table 2).

K-values, initially 16-36%, rose before rejection to 75-92%. In a separate parallel study at RMIT (Cohen and Sumner, unpublished), the major component of the breakdown of nucleotides and purines has been shown to be inosine; murets, therefore, are inosine, rather than hypoxanthine accumulators which accounts for their taste panel acceptability even when K-values approximate 100%. Inosine has not been shown to accentuate any deleterious features of the fish, as has hypoxanthine which is connected with bitter flavour notes.

In two respects the present study adds to the body of circumstantial evidence that warmwater fish keep longer in ice than coldwater species. The value of the present work is that it has studied in some depth one group of fish. For *M. cephalus* the trend in all trials was for fish from warmer waters to have longer ice times than those from cold waters. As well, a very long storage life for *L. subviridis* has been established, the 29-day period being close to the maximum shelf life of 31 days quoted by Poulter, Curran and Disney (1981) for *Nemipterus japonicus*. By contrast, the keeping time of *V. seheli* was short, near to the minimum for tropical species (18 days) for the oily species *Rastrelliger brachyura* and *Scomberomorus commersoni* (Poulter, Curran and Disney, 1981). It may be that the small size (30-50 g) of *V. seheli* has an influence on shelf life. Certainly, small size is equated with high surface area: volume and a major component of spoilage is the external coating of micro-organisms. As well, a small fish has a relatively higher impact from the gut cavity, the other main area of spoilage organisms.

In the five trials storage life of tilapia (*Oreochromis niloticus*) varied between 21 and 27 days (Table 3). Taste panel evaluation established raw and cooked criteria during the storage life. Grade-1 fish (1-4 days old in ice) had bright pinkish, slightly bulging eyes, gills bright maroon with a pleasant "grassy", "vegetable" odour and flesh of firm texture with bright scales. Grade-2 fish underwent progressive changes which saw the eyes gradually become sunken, yellow and cloudy; the gills became brownish and developed a strong, objectionable "fishy" odour, while the flesh felt softer, especially in the belly, and the scales, covered in thick slime, became loose. Taste panelists recorded sweet flavour in Grade 1, with succulent, juicy texture. Gradually, during storage, sweetness was lost and the flavour at rejection was bitter, while also the texture was soft and mushy and the odour strongly ammoniacal.

Objective parameters (Table 4) underwent characteristic changes, with a progressive fall in Torrymeter reading paralleled by rises in bacterial and total volatile nitrogen levels, and in K-value.

Torrymeter readings fall from an initial level of 12, through 9-6 in Grade-2 fish, to 5 or 6 at rejection. A rather narrow range of Torrymeter readings for *O. niloticus* contrasts with a useful range of 16-5 for milkfish (*Chanos chanos*) (Sumner, de la Llanza and Ragasa, 1981) and ranges of 16-7 for several tropical species caught in the Indian Ocean (Hoffman, pers. com.).

Bacterial levels, initially 10^3 - 10^4 /g, rose to 10^5 - 10^7 /g in Grade-2 fish, to 10^5 - 10^8 /g at rejection. Black colonies in Grade 1 ranged from less than 10^2 /g (not detected on 10^2 dilution

Table 1

Shelf lives in ice of mullets from waters of different temperatures

Species	Date	Water Temperature (°C)	Size (kg)	Shelf life in ice (days)	
				Gutted	Ungutted
<i>M. cephalus</i>	1981	9	0.5-1.0	16	20
	1982	8	0.5-1.0	20	21
	1983	14	1.0	21	24
	1981	23	1.0	24	26
	1983	21	1.0	22	26
<i>L. subviridis</i>	1984	26	0.1-0.2	-	29
	1984	26	0.1-0.2	-	29
	1984	26	0.1-0.2	25	28+ ^a / ₂
<i>V. schelzi</i>	1983	28	0.05	-	22
	1984	28	0.05	-	21
	1984	28	0.03	-	22

^a/ Denotes fish supply exhausted while still acceptable to taste panel

Table 2
Torryster, bacterial and K-value changes in ungutted millet during ice storage

Species	Water Temperature (°C)	Initial				Rejection			
		TM	TPC	BBC	K	TM	TPC	BBC	K
<i>N. cephalus</i>	8, 9, 14	9	10^3-10^4	$<10^2$	36	4	10^7-10^9	10^6-10^8	92
	23, 21	10	10^3	$<10^2$	36	4	10^7-10^9	10^5-10^8	87-92
<i>L. subviridis</i>	26	10	10^4-10^5	10^2	23	4	10^8	$.10^7$	75
<i>V. sehelii</i>	28	11	10^4	$<10^2$	16	3	10^8	10^7	83

TM - denotes Torryster reading

TPC - denotes total plate count at 10°C incubation

BBC - denotes black colony count on iron agar

K - denotes % K-value

Table 3
Source, size and shelf life of tilapia stored in ice

Trial	Source	Water Temperature (°C)	Date	Size range (g)	Shelf life (days in ice)
1	Cardona, Laguna de Bay	30	April, 1983	100-150	22+ ^a / ₂
2	Banaue (private farm)	22	April, 1983	75-100	22
3	Banaue (BFAR farm)	16	Dec., 1983	50-100	21
4	La Trinidad (BFAR farm)	16	March, 1984	100-150	23
5	Muñoz (CLSU farm)	28	March, 1984	150-200	27

a/ On day 22, when the supply of ice was exhausted, quality was still acceptable to the taste panel

Table 4
 Torrymeter, bacterial and total volatile nitrogen levels and K-values of tilapia stored in ice

Trial	Grade 1					Grade 2					Grade 3				
	TM	TPC	BBC	TVN	K	TM	TPC	BBC	TVN	K	TM	TPC	BBC	TVN	K
1. Cardona	-	3×10^4	$<10^2$	17	11	-	2×10^7	3×10^5	10	82	-	8×10^9	2×10^8	33	100
2. Banaue (Private)	-	6×10^4	1×10^4	15	22	-	5×10^7	5×10^5	15	92	-	4×10^9	8×10^7	37	100
3. Banaue (BPAR)	12	5×10^4	3×10^3	-	-	8	3×10^7	4×10^5	-	-	5	2×10^8	3×10^7	-	-
4. La Trinidad	11	3×10^3	7×10^2	6	57	8	2×10^7	6×10^5	10	75	6	1×10^8	2×10^7	34	86
5. Muñoz	12	6×10^3	5×10^2	9	44	7	1×10^6	1×10^4	16	78	6	2×10^8	2×10^6	-	83

TM - denotes Torrymeter reading
 TPC - denotes total plate count at 10°C incubation
 BBC - denotes black colony count on iron agar
 TVN - denotes total volatile nitrogen (mg TVN-%)
 K - denotes % K-value

plates) in fish taken from Laguna de Bay at 28°-30°C, to 10^4 /g in fish taken from the rica paddy farm in Banaue (temperature of water 20°-22°C). In Grade 2 black colonies were 10^4 - 10^5 /g rising to 10^6 - 10^8 /g at rejection; throughout the storage period black colonies comprised 1-10% of the total microflora.

Because of their colour reaction on iron agar, black colonies are able to produce sulphides and, though not characterized in the present study, almost all black colonies in fish which is at the end of its ice storage life are either *Alteromonas putrefaciens* or *Pseudomonas* spp. The biochemical activity of these genera has been described by van Sprockens (1977) and, as well as producing sulphides, *A. putrefaciens* and *Pseudomonas* are able to degrade non-protein nitrogen compounds to volatiles like ammonia, and to degrade inosine monophosphate (IMP) to inosine and hypoxanthine (Hx).

Not surprisingly, then, total volatile nitrogen levels rose during storage to over 30 mg TVN-N/100 g flesh at rejection. Similarly, K-values rose from 11-57% in Grade 1 through 75-92% in Grade 2, to 83-100% at rejection. The wide disparity of K-values in Grade-1 fish results from a difference in methodology in the various trials. In Trials 1 and 2 a section of muscle excised from fish immediately after they were captured was frozen quickly in dry ice to minimize breakdown of nucleotides; initial K-values of 11% and 22%, Trials 1 and 2, respectively, therefore reflect nucleotide breakdown from netting of the fish. In Trials 4 and 5, by contrast, fish were placed still alive in a chest containing crushed ice. Undoubtedly struggling took place before the fish died; this higher level of activity is reflected in the relatively higher K-values (44% and 57% in Trials 4 and 5, respectively).

In the present study shelf life of tilapia in ice ranged from 21 to 27 days, a range in line with previous work, Adebola (1981) quoting 21 days, and Disney *et al.* (1971) and Watanabe (1965) 28 days for tilapia from Africa; no information was given on water temperature.

Tilapia acclimatized either in "warm" (35°-37°C) or in "cold" (15°-17°C) and stored in ice had shelf lives of 26 and 22 days, respectively, as judged by both raw (general acceptability) and cooked (flavour) scores (Table 5). These shelf lives approximate those obtained for tilapia from mountain ponds and from lowland ponds (Table 3) in which tilapia from Banaue and La Trinidad kept a few days less than those from the warmer waters of Laguna de Bay and Munoz. The present study reports only two trials in which fish have been acclimatized prior to ice storage; further work is in progress in which bacterial levels, both qualitative and quantitative, will be studied.

Table 5

Sensory scores of tilapia (*O. niloticus*) acclimatized in "cold" (15°-17°C) or "warm" (35°-37°C) water prior to storage in ice

Days in Ice	Coldwater tilapia		Warmwater tilapia	
	Raw score	Cooked flavour	Raw score	Cooked flavour
0	5.0	5.0	5.0	5.0
7	4.6	4.9	4.6	4.8
12	3.6	3.4	4.3	4.3
16	2.7	3.0	2.8	2.5
19	2.3	2.2	3.2	3.0
22	2.0	2.0	3.0	2.8
26	1.2	1.0	2.0	1.8

4. REFERENCES

- Adebona, M.B., Studies on the keeping quality of *Chrysichthys* and tilapia during ice preservation.
1981 In Advances in the refrigerated treatment of fish. Sci.Tech.Froid/Refrig.Sci.Technol.,
Paris, (1981-4):125-33
- Cann, D.C., Bacteriology of shellfish with reference to international trade. In Proceedings of
1977 the Conference on handling, processing and marketing of tropical fish. London, Tropical
Products Institute, pp. 377-94
- Chervinski, J., Environmental physiology of tilapias. ICLARM Conf.Proc., (7):119-28
1982
- Chimits, P., The tilapias and their culture: a second review and bibliography. FAO Fish.Bull.,
1957 10(1):1-24
- Conway, E.J., Microdiffusion analysis and volumetric error. London, Crosly, Lockwood and Son,
1968 467 p.
- Disney, J.G., et al., Quality assessment in Tilapia species. In Fish inspection and quality
1971 control, edited by R. Kreuzer. London, Fishing News (Books) Ltd.
- FAO, Actas del simposio sobre acuicultura en América Latina. FAO Inf.Pesca., (159) 3 vols: pag. var.
1977
- Jensen, M.N. and E. Schultz, Utilisation of iron agar in determining the freshness of wet fish.
1980 Dan.Vet.Tidskr., 63:314-8
- Lima dos Santos, C.A.M., The storage of tropical fish in ice - a review. Trop.Sci., 23:97-127
1981
- Philippart, J.-C. and J.-C. Ruwet, Ecology and distribution of tilapias. ICLARM Conf.Proc., (7):
1982 15-59
- Poulter, R.G., C.A. Curran and J.G. Disney, Chill storage of tropical and temperate water fish -
1981 differences and similarities. In Advances in the refrigerated treatment of fish.
Sci.Tech.Froid/Refrig.Sci.Technol., Paris, (1984-1):111-24
- Shewan, J.M., The bacteriology of fresh and spoiling fish and the biochemical changes induced by
1977 bacterial action. In Proceedings of the Conference on handling, processing and
marketing of tropical fish. London, Tropical Products Institute, pp. 51-66
- Spreekens, K.J.A. van, Characterisation of some fish and shrimp spoiling bacteria. Antonie van
1977 Leeuwenhoek, 43:283-303
- Sumner, J.L., J. de la Llama and R. Ragasa, Correlation between Torrymeter reading and bacteri-
1981 ological counts of milkfish (*Chanos chanos*). Fish.Res.J.Philipp., 6(2):11-3
- Trewavas, E., Tilapias: taxonomy and speciation. ICLARM Conf.Proc., (7):3-13
1982
- Uchiyama, H. and H. Uchiyama, Simple and rapid method for estimating the freshness of fish. Bull.
1970 Tokai Reg.Fish.Res.Lab., (61)
- Watanabe, K., Handling and keeping quality of ice-d Kariba bream, *Tilapia mortimeri* Trewavas (syn
1965 *T. mossambica* Peters). Fish.Res.Bull.,Zambia, 4:59-64

QUALITY CHANGES IN BRACKISHWATER PRAWNS
(*Penaeus monodon*) DURING STORAGE AT AMBIENT TEMPERATURE,
IN ICE AND AFTER DELAYS IN ICING

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ABSTRACT

Brackishwater prawns (*Penaeus monodon* Fabricius) spoiled within 16 hours at ambient temperature; in 17 days when stored in ice; and 16, 13 and 11 days when icing was delayed for 4, 8 and 12 hours respectively. Trimethylamine and total volatile nitrogen levels fluctuated the storage period and were regarded as poor indices of quality. Bacterial counts were within the range 10^6 - 10^9 /g on rejection and the principal spoilage organisms were *Pseudomonas* spp. and *Alteromonas putrefaciens*. Nucleotide breakdown followed the pathway ATP to ADP to AMP to IMP to inosine and hypoxanthine and the rate of decomposition varied for each storage condition. K-values gave good correlation with storage time; as well as cooked flavour and raw acceptability scores.

1. INTRODUCTION

In the Philippines there has been a rapid expansion in brackishwater production of prawns over the past decade (Reilly, Bernarte and Dangla, 1984). The main research efforts associated with the monoculture of prawns have focused on factors to stabilize and improve production and yield. In spite of their popularity as a food and value for export, relatively little attention has been given to post-harvest handling of brackishwater prawns. Differences exist in environmental growth conditions in ponds when compared to the marine and coastal areas, such as salinity, temperature, microbial population, organic and inorganic matter. Data on how these environmental growth conditions affect the iced storage life is scarce.

Traditionally prawns are harvested at night during high or low tide (Reilly, Bernarte and Dangla, 1984) and are sometimes maintained at ambient temperature for 8 hours or longer prior to icing. Handling practices vary from packing prawns in rattan baskets or metal tubs (baneras) uniced to improper icing in polystyrene boxes. No data are available on the effects of delayed icing or prolonged exposure of brackishwater prawns to high temperatures.

The objective of our study was to determine the overall effect of delays in icing on the subsequent storage life and quality of brackishwater prawns (*Penaeus monodon* Fabricius).

2. METHODS

2.1 Sampling

Three trials were carried out on brackishwater prawns for coastal areas around Manila Bay. All samples were alive when taken and the sizes varied from 20-30 prawns per kilo. Immediately on capture one batch of samples was iced and three lots maintained at ambient temperature (20-30°C) for 4, 8 and 12 hours respectively before icing in insulated fish boxes. Initial samples for nucleotide analysis were frozen in dry ice immediately after harvesting. Fish boxes were kept in a chill room at 5°C throughout the storage period and ice replenished when required. Fifteen (15) prawns were taken for chemical, and sensory microbiological analyses on each sampling day.

2.2 Chemical Analysis

Trimethylamine (TMA) and Total Volatile Nitrogen (TVN) were determined on trichloroacetic acid extracts from 30 g of prawn flesh by the Conway microdiffusion technique (Conway, 1968). K-values

and ATP breakdown were determined by the method of Kobayashi and Uchiyama (1970). pH was measured with a portable Camlab Digital pH meter after homogenizing 2 g of prawn flesh in 10 ml distilled water for 30 seconds. Weight loss during storage at ambient temperature was determined by weighing 5 prawns every 4 hours calculated on a wetweight basis.

2.3 Microbiological Analysis

Standard plate counts (SPC) and H_2S producers count at 20°C were carried out using iron agar (Jensen and Schultz, 1980). Bacterial isolates during storage were identified according to the scheme of Cowan (1974) and gram negative bacteria using the API 20E system (API International, Geneva, Switzerland).

2.4 Sensory Analysis

Raw acceptability and cooked sensory attributes were evaluated by six trained taste panelists using the score cards in Tables 1 and 2. Cooked samples were steamed for 20 minutes wrapped in aluminium foil.

3. RESULTS AND DISCUSSIONS

The changes in SPC's and H_2S producers counts are presented in Figures 1 and 2. At ambient temperature, there was a rapid increase in bacterial numbers from 10^4 - 10^5 /g with 16 hours. The changes in the spoilage microflora are presented in Figure 3 showing *Pseudomonas* and *Enterobacteriaceae* dominating at rejection. The SPC of prawns immediately iced showed a gradual increase over the storage period from 10^4 - 10^5 /g, whereas SPC's of samples held at ambient temperatures for 4, 8 and 12 hours initially decreased after 3 days and then increased to 10^7 - 10^9 /g after 14-17 days. This is due to a decrease in the mesophilic flora giving way to psychrophiles during iced storage. The H_2S producers were detected after 7 days and increased to 10^5 - 10^6 /g (Figure 2). *Alteromonas putrefaciens* and *Pseudomonas* dominated at rejection (Figure 3).

Based on cooked flavour scores prawns were rejected by taste panelists after 17, 16, 13 and 11 days for samples which were immediately iced and delays of 4, 8 and 12 hours respectively, however prawns were rejected sooner based on raw acceptability scores (Figure 4). Rejection of raw samples was based on ammoniacal and sulphidic offensive odours, soft texture and discolouration. Cooked samples were rejected principally on account of fishy, sulphidic, ammoniacal flavours and odours. Prawns held at ambient temperature were rejected after 16 hours due to complete discolouration with a pinkish cooked appearance, strong ammoniacal odours and very soft texture. Prawns lost up to 15% of their body weight after 16 hours, although 10% was regained with subsequent storage in ice. It is of interest to note that sulphidic odours were not detected in samples stored at ambient temperature and that H_2S producing bacteria were not isolated during this period. The raw acceptability and cooked flavour scores gave significant correlation with days storage at the 1% level (Table 3). Shelf life based on cooked flavour scores shows that for every hour delay in icing approximately 0.5 day storage in ice is lost. More serious losses in terms of shelf life occur in fish (Barile *et al.*, these proceedings). However, the overall deterioration in raw acceptability means that prawns lose their value as prime quality (raw frozen head-on) for export after approximately 8 hours at ambient temperature.

Both TMA and TVN are of doubtful value as quality indices of some tropical fish and shellfish (Lima dos Santos, 1981; Reilly, Barnarte and Dangla, 1984). The values fluctuated during ambient temperature storage and subsequent storage in ice, with highest levels obtained as delays in icing were extended (Table 4). The limit of acceptability of 5 mg% (TMA) suggested by Montgomery, Sidhu and Vale (1970) was never reached, and 30 mg% (TVN) was exceeded within 7-10 days. The fluctuations in levels of TMA and TVN are probably due to the washing effects of ice during storage (Cobb *et al.*, 1976).

Two pathways have been suggested for the breakdown of ATP in prawns and shrimp. One involves the deamination of AMP to IMP, while the second involves the dephosphorylation of AMP to adenosine. The degradation of adenosine triphosphate (ATP) in brackishwater *P. monodon* followed the pathway: ATP to adenosine diphosphate (ADP) to adenosine monophosphate (AMP) to inosine monophosphate (IMP) to inosine and hypoxanthine ($HxR + Hx$) which is in agreement with the route of ATP breakdown in brown shrimp *Penaeus aztecus* (Flick and Lovell, 1972). However, Cheuk, Finne and Nickelson (1979) suggested that the principal pathway for nucleotide degradation in shrimp held on ice was through adenosine rather than IMP.

The progression of changes in nucleotide composition of brackishwater prawns during storage at ambient temperature and in ice/icing delays are shown in Figure 5. The initial concentration of ATP (4.0 μ mole/g) decreased rapidly at ambient temperature and was not detected after 3 days in prawns which were immediately iced. Prawns did not exhibit any of the characteristics associated with rigor mortis commonly associated with post mortem changes in fish and nucleotide breakdown. ADP was present in newly harvested prawns at a level of 3.0 μ mole/g which decreased during iced and ambient temperature storage. The level of AMP increased to 4.5 μ mole/g during the first 7 days on

ice followed by a rapid decrease (Figure 5). An increase in AMP was also found at ambient temperature up to 4 hours followed by a gradual decrease to 1.7 μ Mole/g after 12 hours. IMP followed a similar pattern with a maximum concentration of 6.3 μ Mole/g after 8 hours at ambient temperature. Inosine and hypoxanthine were not separated but measured as one peak. The levels gradually increased with storage time with a rapid increase at the latter stages of storage on ice and at ambient temperature. HxR and Hx concentration appear to be closely related to the disappearance of the other nucleotides and the increase in bacterial population. The presence of AMP up to the end of storage suggests a different rate of ATP breakdown in prawn than in fish. After delays in icing and subsequent storage in ice nucleotide degradation showed different patterns, probably due to the effect of temperature on enzyme activity (Figure 5). Further work needs to be carried out to clarify the effect of delays in icing on nucleotide decomposition in prawns. Nucleotide decomposition as measured by the freshness index or K-value (Saito, Arai and Matsuyoshi, 1959) reached 67% after 16 hours at ambient temperature. K-values in excess of 60% are associated with spoiled fish (Ehira, 1976). The rejection value for prawns immediately iced was 36% and samples held for 4, 8 and 12 hours were 45, 64 and 71% respectively. The rejection value of 36% is very low when compared to fish immediately iced (Barille *et al.*, these proceedings) which again suggests a different rate of ATP breakdown.

The pH values ranged from 7.1-8.1 (Figure 7) and higher values were found with extended delays in icing. The increases in pH values can be attributed to the higher levels of volatile nitrogen compounds produced by microbial and tissue enzymes in prawns.

4. REFERENCES

- Cheuk, W.L., G. Finne and R. Nickelson, Stability of adenosine deaminase and adenosine monophosphate during ice storage of pink and brown shrimp from the Gulf of Mexico. J.Food Sci., 44:1625-8
- Cobb, B.F., *et al.*, Effect of ice storage on microbiological and chemical changes in shrimp and melting ice in a model system. J.Food Sci., 41:29-34
- Conway, E.J., Microdiffusion analysis and volumetric error. London, Crosby, Lockwood and Son 1968
- Cowan, S.T., Cowan and Steel's manual for the identification of medical bacteria. Cambridge, Cambridge University, 2nd ed.
- Ehira, S., A biochemical study on the freshness of fish. Bull.Tokai Reg.Fish.Res.Lab., (86) 1976
- Flick, G.L. and R.T. Lovell, Post-mortem biochemical changes in the muscle of gulf shrimp, *Penaeus setiferus*. J.Food Sci., 37:609-11
- Jensen, M.H. and E. Schultz, Utilization of iron agar in determining the freshness of wet fish. 1980 Dan.Vet.Tidsskr., 63:314-8
- Kobayashi, H. and H. Uchiyama, Simple and rapid method for estimating the freshness of fish. 1970 Bull.Tokai Reg.Fish.Res.Lab., (61)
- Lima dos Santos, C.A.M., The storage of tropical fish in ice - a review. Trop.Sci., 23:97-127 1981
- Montgomery, W.A., G.S. Sidhu and G.L. Vale, The Australian prawn industry. CSIRO Food Preserv.Q., 1970 30:21-7
- Reilly, A., M.A. Bernarte and E. Dangle, Storage stability of brackishwater prawns during processing for export. Food Technol., Aust., 36:283-6
- Saito, T., K. Arai and M. Matsuyoshi, A new method for estimating the freshness of fish. Bull.J. Soc.Sci.Fish., 24:749-50 1959

Table 1

Freshness score sheet for brackishwater *P. monodon*
Raw prawns

Score	Odour	General Appearance	Texture
10	Seaweed, characteristic of the species (crab-like)	Head bluish gray and completely attached to body; body bluish gray with dark blue and yellow bands, tail with bluish yellow stripes	Firm, elastic, hard shell, tight body
9	Slightly seaweed, fresh cut grass	Head grayish black and still attached to body; body grayish black with bands on the tail grayish black to black with light yellow stripes	Fairly firm, slightly elastic
8	Slight characteristic odour of the species watery	Head gray with slight blackening; body gray with bands of lighter shade; tail blackish with light yellow stripes	Slightly soft body/shell
7	Neutral to bland		
6	Slightly ammoniacal	Head with blackening near eye region slightly loose carapace with tiny white spots, grayish body with black spots on the band; tail bluish-black with dirty yellow stripes	Slightly soft body/shell
5	Slightly urinal, slightly fishy	Head almost completely black and slightly loose with white spots; body grayish with light brownish bands; tail brownish to brownish black	
4	Fish offensive, sulphid	Head completely black and loose; reddish gray body with brown and black bands, tail black	Soft body and shell
2	Ammoniacal, sulphid	Head completely black and slightly detached from body; body reddish gray with alternating black and brown bands tail black	Body sponge-like
1	Strong sulphid, uric		Mushy
0	Faecal, strong ammoniacal	Head completely black and slightly to completely detached; body grayish with dark brown and black bands with black spots all over the body; tail black	Very soft, papery shell, mushy

Table 2

Freshness score sheet for brackishwater *P. monodon*
Cooked prawns

Score	Odour	Flavour	Texture
10	Crabby, characteristic odour of species, sweet, seaweedy	Sweet, meaty, crabby characteristic odour of species	Meaty, juicy, tender firm
9		Slightly sweet to bland, slightly bitter aftertaste	
8	Slightly crabby, sweet, seaweedy	Slightly sweet to bland, slight bitter itchy aftertaste	Firm, chewy
7	Fresh cut grass, slightly crabby, slightly ammoniacal, very slight urinal		
6	Slightly ammoniacal, slightly sweet characteristic odour	Slightly sweet, almost neutral, bland	Slightly firm to dry
5	Strong ammoniacal	Bland, neutral to slightly fishy	
4	Strong ammoniacal, strong urinal	Bland to fishy, offensive sulphidic	
2	Strong ammoniacal, strong urinal		Dry, lumpy
0	Faecal, putrid, strong urinal	Strong fishy, bitter, sulphidic, itchy aftertaste	Soft, dry, lumpy

Table 3

Regression statistics for the change in sensory scores of raw and cooked samples of prawns with time of storage

Storage Condition	Raw Samples			Cooked Samples		
	a	b	r	a	b	r
Mesophilic	9.84	-0.31	-0.9094	9.6	-0.335	-0.7571
0-h delay in icing	8.60	-0.316	-0.9713	8.11	-0.258	-0.961
4-h delay	8.32	-0.311	-0.9477	9.33	-0.393	-0.9707
8-h delay	7.76	-0.275	-0.9909	7.99	-0.316	-0.9877
12-h delay	6.84	-0.328	-0.9850	7.44	-0.377	-0.9927

a = y-intercept b = slope of the line
r = coefficient of correlation

Table 4

Changes in TMA and TVN during iced storage and after 4, 8 and 12 hours at ambient temperature

Days storage	Delays Before Iced Storage							
	0 hour		4 hours		8 hours		12 hours	
	TMA	TVN	TMA	TVN	TMA	TVN	TMA	TVN
	mg%	mg%	mg%	mg%	mg%	mg%	mg%	mg%
0	0.71	7.52	0.72	26.62	1.04	16.20	1.01	17.75
3	0.32	25.46	1.01	8.68	2.36	21.53	1.2	66.5
7	2.17	16.78	2.14	14.47	0.43	28.83	-	-
10	0.90	32.9	0.78	31.83	-	-	0.2	72.3
14	0.46	46.3	2.6	19.67	6.8	43.4	2.6	66.5
17	1.36	27.78	1.44	36.17	4.68	82.46	-	-

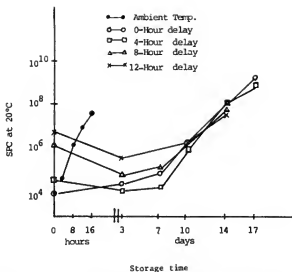


Figure 1 Changes in SPC at 20°C at ambient temperature and during iced storage after delays in icing

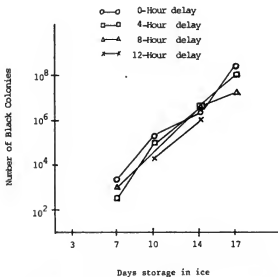


Figure 2 Changes in H_2S producers count at 20°C after delays in icing

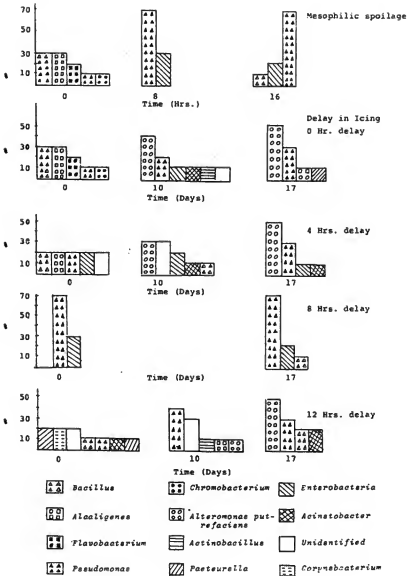


Figure 3 Changes in the microflora of prawns during iced storage with delays

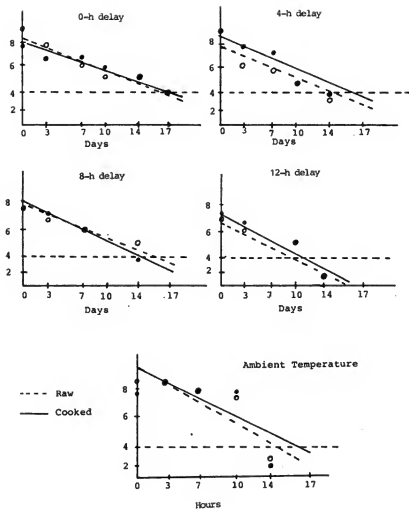


Figure 4 Correlation between cooked flavours and raw acceptability scores with storage time after delays in icing

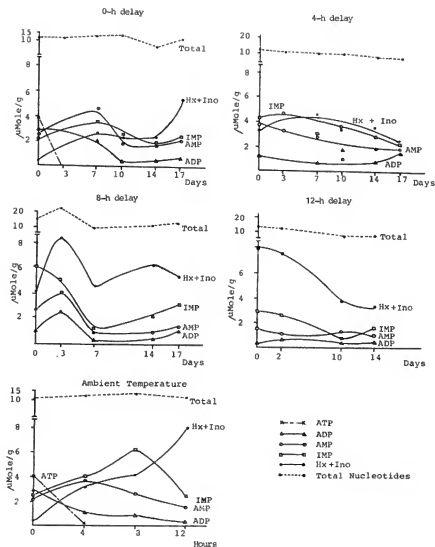


Figure 5 Nucleotide degradation in brackishwater prawns stored at ambient temperature and during ice storage with delay

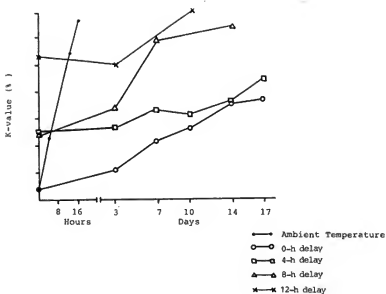
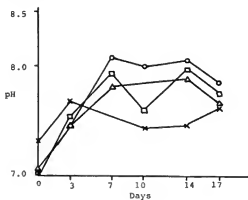


Figure 6 Changes in percent K-value of brackishwater prawns at ambient temperature and during iced storage



Figures 7 Changes in pH values of brackishwater prawns during iced storage

A PRELIMINARY STUDY ON THE SEASONAL VARIATION IN THE STORAGE
PATTERN AND PROXIMATE COMPOSITION OF ICED SPOTTED SARDINE
(*Sardinella eirm*)

by

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ABSTRACT

Seasonal changes in the proximate composition and storage life of sardines (*Sardinella eirm*) was studied over a period of nine months (June-February). Results indicated that there was no considerable seasonal variation in the proximate composition except a slight variation in fat content. When fish mature the fat content drops from 3.0-1.5%. No variation was found in iced storage life, which was about 5 days. TMA and TVN values were not good indices of fish quality.

1. INTRODUCTION

Spotted sardine (*Sardinella eirm*) is caught by gillnet in the coastal waters of Sri Lanka throughout the year. Most of the fish caught is not utilized in the wet form due to problems in transportation to market centres. The surplus of fish is converted to salted and dried fish in fishing areas. The Institute of Fish Technology has initiated research work with the view to introduce new products from spotted sardine such as vacuum packed fish, smoked fish and pickle cured fish. The fish is to be purchased from Northern and Eastern regions and has to be kept on ice for a few days prior to processing. Laboratory analysis revealed that fish caught during certain seasons of the year spoil faster. In Sri Lanka, there are no records of studies on the storage life of spotted sardine and thus the present work would be of importance to the processing industry.

It has been reported that the chemical composition of fish varies seasonally (Love, 1970) and the storage period of fish changes according to the individual constituents, especially with fat content. The composition of fish is influenced by the age, sex, maturity stages and feeding habits of the fish (Love, 1970; Rao, 1971). Hence it is important to study the factors which determine the storage period of fish.

This study was undertaken to examine whether there is any seasonal variation in shelf life and proximate composition of fish stored on ice and to correlate these with the maturity of the fish.

2. MATERIALS AND METHODS

Freshly caught fish (*Sardinella eirm*) was purchased from landing sites in Negombo in the west coast of the island and was iced immediately. Fish was stored at 0°C throughout the period of the experiment. The deterioration in the quality was assessed both chemically and organoleptically.

Ash, moisture and protein content of the fish was determined by AOAC method (AOAC, 1980). Fat content was determined by Bligh and Dyer method (Hansen and Olley, 1963).

The Cooway-Byrnes method was used to determine total volatile nitrogen and trimethylamine content (Beatty and Gibbons, 1937).

The fish was served to the members of the taste panel who judged for odour, flavour and overall quality. The 0-10 hedonic scale was used where the limit of acceptability is 4.

In each month the stage of maturity of fish that was subjected to analysis was examined using the following classification (Reje, 1971).

- Stage I Immature - Immature gonads which are small and colourless.
- Stage II Developing - Immature gonade which are slightly larger than in the previous stage with a few oocytes
- Stage III Maturing - Ovaries with ova visible, turgid, yellow in colour with granular appearance. Ovaries occupy 70% of the body cavity.

- Stage IV Maturing - Ovaries occupy the entire body cavity and containing large opaque ova.
- Stage V Mature - Ovaries orange yellow in colour, prominent blood vessels ramifying on the surface. Ova large and translucent.
- Stage VI Spawning - Ovaries with oozing ova which are transparent. Eggs easily extruded by slight pressure on the flanks.
- Stage VII Spent - Ovaries flaccid, occupying approximately half the length of the body cavity, reddish and bloodshot.

3. RESULTS AND DISCUSSIONS

The results of the proximate composition (Figure 1) show a clear interrelationship of the main constituents of fish flesh. A remarkable inverse relationship was noted between the fat content and moisture content. The same pattern has been reported for sardines. There seems to be no considerable seasonal variation of the individual constituents. Only a slight variation in fat content was observed. Highest fat content (3.2%) was found in June while the lowest value (1.5%) was recorded in July and November. It is apparent that when fish mature fat content drops from 3.2-1.5% (Figure 2). Several workers have shown that there is a depletion of fat in fish flesh during the maturation of gonads (Rao, 1971; Love, 1970; Lovern and Wood, 1937). Rao also stated that during maturation the fats which are stored in various organs are transferred to maturing gonads. Present results showed (Figure 2) that the highest fat content could be found only at stage II (developing). Immature fish also contained lower amounts of fat. Our results showed that spawning takes place in September and that there was no change in fat content in the pre-and post-spawning periods.

Results of the taste panel scores (Table 1) showed that in each month, quality of the fish deteriorated after 5-6 days of iced storage. There was no considerable seasonal variation in the storage life except in the month of August. The quality of the fish caught in the month of August was good even after 7 days of iced storage as compared to fish caught in other months. This could be attributed to the better initial quality of the fish. Therefore it was concluded that fish could be maintained for 5 days in ice in an acceptable condition.

TVN values (Figure 3) did not indicate considerable variations seasonally except in fish caught in September where TVN values are very low (from 14-24 mg %). Tanikawa (1935) has suggested that 30 mg % be the upper limit of acceptability for fresh fish. Except in June, July and August TVN values do not exceed the level of 30 mg % during the storage period. But TVN values were very low even on the 6th day, when the panel rejected the fish as not fit for human consumption. TMA values too do not seem to indicate the spoilage pattern of fish (Figure 4). Therefore TVN and TMA values of *Sardinella sirm* do not seem to be useful as an index of freshness.

4. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1980 AOAC
- Beatty, S.A. and N.E. Gibbons, The measurement of spoilage in fish. *J.Biol.Board Can.*, 3:77-91 1937
- Hansen, S.W. and J. Olley, Application of the Bligh and Dyer method of lipid extraction to tissue 1963 homogenates. *Biochem.J.*, 89
- Love, R.M., The chemical biology of fishes, London, Academic Press, pp. 225-57 1970
- Lovern, J.A. and H. Wood, Variation in the chemical composition of herring. *J.Mar.Biol.Assoc.U.K.*, 1937 22:281-93
- Raja, B.T.A., On the maturity stages of Indian oil-sardine *Sardinella longiceps* Val., with notes 1971 on incidence of atretic follicles in advanced ovaries. *Indian J.Fish.*, 13(1-2):27-47
- Rao, T.A., Fat and water contents of the muscle and ovary during the maturation cycle of 1971 *Pseudociaena areolaris* (Bloch) and *Johnius carutta* (Bloch). *Indian J.Fish.*, 14(1-2):293-7
- Tanikawa, E., Studies on measuring freshness of fish and shellfish meal. *J.Soc.Ind.Fish., Japan*, 1935 3:267-92

Table 1

Overall taste panel scores for iced
Sardinella sirm

Months	Days	Overall scores						
		1	2	3	4	5	6	7
June		8.0	7.0	6.5	8.0	5.0	4.0	3.0
July		8.0	7.5	8.0	6.0	5.0	4.0	3.0
August		9.0	8.0	7.0	7.0	6.5	8.0	5.0
September		9.0	8.0	7.5	6.0	5.0	4.0	3.0
October		8.0	8.0	7.0	6.0	4.5	4.0	3.0
November		8.5	8.0	8.0	7.0	5.0	4.0	3.0
December		8.0	8.0	7.5	8.0	5.0	3.0	3.0
January		7.0	7.0	8.5	6.0	5.0	4.0	2.0
February		8.5	8.0	7.0	5.5	5.0	3.0	3.0

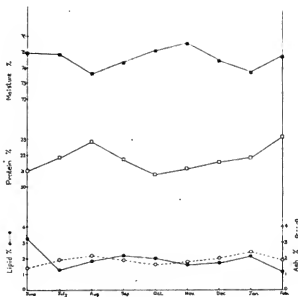


Figure 1 Seasonal variation in the four principal constituents of *Sardinella sirm*

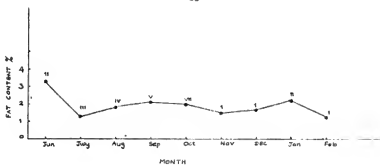


Figure 2 Seasonal variation in fat content at different stages of maturity

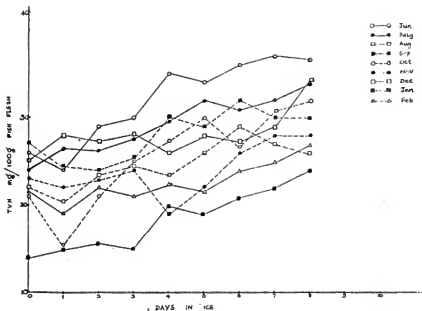


Figure 3 TVN values of spotted sardine stored in ice

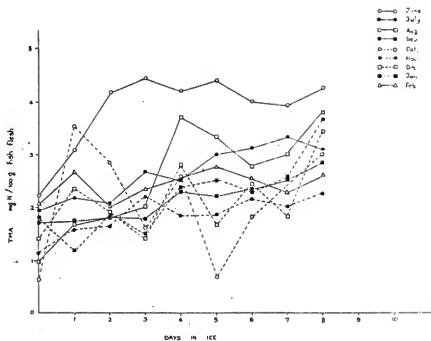


Figure 4 TMA values of spotted sardine stored in ice

STUDIES ON SEMI-COMMERCIAL TRIALS ON THE USE OF PARABEN-ICE
FOR THE PRESERVATION OF FRESH FISH

by

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ABSTRACT

Preservation of fresh fish with propyl paraben incorporated ice (Paraben-ice) on a semi-commercial scale was tried on three species of marine fishes namely *Nemipterus japonicus* (threadfin bream), *Johnopsis* spp. (croaker), *Tachysurus* spp. (marine catfishes) and trash fish (mixture of fish species consisting of *Cyanoglossus* spp., *Leiognathus* spp., *Saurida tumbil* and *Grausoplites* spp.). The results indicated that paraben-ice could extend the shelf life of *Johnopsis* spp., *Tachysurus* spp., and trash fish by ten days over their respective controls preserved in ordinary ice. In the case of *Nemipterus japonicus*, the extension of shelf life was only 4-6 days over that of control.

1. INTRODUCTION

About 65% of the annual fish catch in India is consumed fresh or after icing. The shelf life of an iced fish, in general, may not exceed 10-12 days under ideal conditions and as such, this period is inadequate for long distance transportation of fish. Therefore, further extension of shelf life by another 8-10 days could be of great practical significance, particularly for underdeveloped and developing countries, where fast moving refrigerated transport systems to distant markets are lacking. While working towards this objective, Anand and Setty (1981) found that propyl paraben, among various other preservatives studied, was most effective in preventing the growth of almost all the groups of fish spoilage bacteria. In another study, workers at the College of Fisheries, Mangalore, found that the shelf life of fish could be extended by 1-3 weeks over that of control by using 0.1% propyl paraben incorporated ice (paraben-ice).

In the present study, the effectiveness of paraben-ice in extending the shelf life of common Indian fish has been tried on a semi-commercial scale and their shelf lives have been assessed using both sensory and non-sensory methods.

2. MATERIALS AND METHODS

The experiments were carried out on the following marine fishes: *Nemipterus japonicus* (threadfin bream), *Johnopsis* spp. (croaker), *Tachysurus* spp. (marine catfish) and a mixture of trash fishes comprising *Cyanoglossus* spp. (soles), *Leiognathus* spp. (silverbelly), *Saurida tumbil* (lizard fish) and *Grausoplites* spp. (rough flathead fish). The fish were caught at depths of 20-25 fathoms by trawl net in the inshore waters of Arabian Sea off Mangalore.

The experimental preservative used was paraben-ice, with ordinary ice as the control. Paraben-ice was prepared by incorporating n-propyl-p-hydroxy benzoate, obtained from BDH Chemicals Ltd., Poole, England, into water at 0.1% level. Immediately after hauling, the fish were washed with clean seawater and each species or group of species were divided into two lots, one of which was preserved in ordinary ice (control) and the other in paraben-ice (experimental preservative). The samples were then brought to the laboratory in insulated high density polythene boxes provided by the FAO and stored at 2°C ($\pm 1^\circ\text{C}$). Ice or paraben-ice was added periodically to the lots concerned to replace the melted ice, so as to maintain their temperature.

Storage trials were carried out on two batches of threadfin bream and on one batch of the other three groups.

Fish samples were taken on the day of catching and on alternate days thereafter. The samples were analysed for total bacterial counts using the methods of the APHA (1976), for trimethylamine (TMA) and total volatile nitrogen (TVN) by the method of Beatty and Gibbons (1937) and organoleptic evaluation by trained panellists, using the 10 point hedonic scale suggested by Connell (1975). An organoleptic score of 4 and below was taken as not acceptable.

3. RESULTS AND DISCUSSION

Results of the storage studies in respect of the two batches of *Nemipterus japonicus* (threadfin bream) are depicted in Figures 1 and 2. Although in both cases, the fish were freshly caught and preserved immediately in ice/paraben-ice, there was a slight difference in their initial quality based on organoleptic evaluation. Batch I was found to have slightly lower initial quality than batch II. A good correlation was observed between the organoleptic assessment and total bacterial load (Figures 1 and 2). The increase in the bacterial load and the decrease in organoleptic score in the case of parabens preserved fish was much more gradual compared to those in the control. The control lot was found to be acceptable up to 10 days in batch I and up to 12 days in batch II and they were organoleptically unacceptable on the 12th and 14th days respectively. The corresponding values for TPC on the day of spoilage had also reached the limit of acceptability (8-10 million bacteria per gram) in both the catches. The fish stored in paraben-ice were found to be organoleptically acceptable up to the 16th day on both the batches and were spoiled only on the 18th day. The corresponding values for TPC on the day of spoilage had also reached the limit of acceptability. TMA values showed a gradual increase over the period of storage in both the batches. However, the increase was more rapid in the control, particularly in batch II. On the day of spoilage, both the control and paraben-ice preserved fish, the maximum values for TMA were in the range of 2.5-3.5 mg/L. The increase in shelf life obtained due to the use of paraben-ice was six and four days in batches I and II respectively. It is apparent from the figures that the fish preserved in ordinary ice in batch I became unacceptable on the 12th day and in batch II on the 14th day, which was presumably due to the difference in their initial quality. However, a corresponding difference in the shelf life of fish stored in paraben-ice was not observed. This is probably due to the decrease in the effective concentration of the preservative on the bacterial cells towards the later stages of storage as the bacterial numbers would have increased progressively with time.

The results of the storage studies with respect to *Johnius* spp. (croaker) are presented in Figure 3. It is clear that the correlations between TPC, TMA and organoleptic assessment were as good as those in the case of threadfin bream. However, the extension of shelf life due to the use of paraben-ice was of the order of ten days, unlike only four to six days as in the case of threadfin bream. The TPC and TMA values on the day of spoilage of fish, as judged by organoleptic assessment, were also similar to those of threadfin bream.

It is interesting to note that both croaker and threadfin bream spoiled around the 12th day when stored in ordinary ice, whereas in the case of fish stored in paraben-ice, croaker spoiled on the 22nd day and threadfin bream on the 18th day. In both these fishes there were no significant differences in the ultimate values of TMA and TPC on the day of spoilage, the only difference being in the organoleptic judgement of both the fishes. This gave rise to a doubt whether psychological factors play an important role in organoleptic judgement, which was mainly influenced by colour, appearance and texture of the fish. Otherwise, this observation is difficult to explain.

The results of storage studies on catfish are shown in Figure 4. They were also almost similar to those relating to croaker. The extension of shelf life due to the use of paraben-ice in this case was ten days over that of fish preserved in ordinary ice. However, the shelf life of catfish in ordinary ice was found to be sixteen days, as against ten days in the case of croaker. Paraben-ice enabled the catfishes to remain in good condition for twenty-six days, as against twenty days in the case of croaker.

As in the case of croaker and catfishes, the paraben-ice extended the shelf life of trash fish also by ten days over that of the control (Figure 5). The shelf life of trash fish both in ordinary ice and paraben-ice appears to be similar to that of catfish and there were no significant differences in the final values of TPC and TMA obtained when rejected.

The TVN values showed a gradual increase during the storage period in all the four groups of fishes (Figure 6) and reached the limit of acceptability (30 mg/L) on the day of spoilage in threadfin bream of both the batches preserved in ordinary ice, and of batch I preserved in paraben-ice, and in croaker preserved in paraben-ice. In all other cases, the TVN values remained in the range of 23-26 mg/L, except in the case of trash fish.

Among the various sensory and non-sensory methods used for assessment of keeping quality of ice preserved fish, organoleptic assessment, by far, seems to be the most widely accepted commercial method. Therefore, this method has been taken as standard in this study, and the results obtained for non-sensory methods like total bacterial counts, trimethylamine (TMA) and total volatile nitrogen (TVN) have been compared with this.

Although total bacterial counts do give an idea on the quality of ice preserved fish and much work has been done in this respect, there seems to be no definite standard fixed for using total bacterial counts alone or in conjunction with organoleptic evaluation, as a criterion to judge the fish as acceptable or not. Changes detectable to human senses occur only when the bacterial load reaches 1 to 10 million per gram. Thatcher and Clark (1968) have observed that decomposition of

the material generally becomes evident in foods having a bacterial load of 10^6 - 10^8 /g. Shewen (1962) found that the bacterial counts of cod and haddock stored in ice reach maximum values of 10^7 - 10^8 /cm² of skin after 10-12 days. These fishes were generally considered stale after twelve days and become inedible after 3-4 days of further storage. Based on total bacterial load and organoleptic evaluation, Anand (1976) categorized those samples whose bacterial count fell between 2-5 million per gram as "fairly good" and those between 5-8 million per gram as acceptable, since a faint off odour was evident at this stage, and bacterial count between 8-10 million per gram as "not acceptable". In this study also, bacterial counts around 10^7 /g appear to agree with the organoleptic assessment in judging the fish as "unacceptable".

In fixing the critical value of TMA for the edibility of fish, the situation is an ambiguous one. Beatty and Gibbons (1937) have suggested a TMA content of 4-6 mg% as the critical value for the edibility of fish, while Connell (1975) has suggested 10-15 mg% for temperate fish. There is also wide variation in critical values suggested for individual species, like 5-7 mg % for herring (Sigurdsson, 1955) and 1-5 mg % for haddock (Castell and Triggs, 1955).

In the present study, the TMA values never went beyond 3.5 mg % in any of the fish examined on the day of spoilage. Therefore, the suggestion of Castell and Triggs (1955) mentioned above appears to be more applicable to fishes studied here. In an unpublished study by the College of Fisheries TMA for ice stored croaker never exceeded 4 mg %, even on the 15th day. Therefore, it is understandable that the TMA values in all four cases were in agreement with the organoleptic assessment, as well as the total plate count.

The critical value for edibility of fish in respect of TVN has been suggested to be 30 mg % by several workers (Tillman and Otto, 1924; Shimizu, 1925; Yemura, 1933; Tanikawa, 1935). Kimura and Kiamakura (1934) recommended TVN levels of 10 mg % or less for fresh fish, 20-30 mg % for beginning of spoilage and over 30 mg % for spoiled fish. However, Glaesman and Rochwerger (1929) recommended 20 mg % as the critical value for edibility. In the present study, the TVN values in most cases were in the range of 23-26 mg % on the day of spoilage, and this agrees with the recommendation of Glaesman and Rochwerger (1929). By and large, TVN has been considered to be an unsatisfactory indicator of spoilage (Ferber, 1965).

The extension of shelf life achieved over that of control by the use of perben-ice was of the order of 4-6 days in the case of threadfin bream and 10 days in the case of croaker, catfish and tresh fish. Anand (1976) in his studies on croaker found that this fish when dipped in propyl paraben solution for 30 min and preserved in ordinary ice could show an extended shelf life of two weeks over that of control. However, this study was conducted on a laboratory scale using 2 kg of fish per batch. Similar experiments with croaker on a semi-commercial scale (25 kg/batch), found that the fish dipped in 0.1% paraben solution for 15-30 min and preserved in ordinary ice, kept well for only 6-7 days over that of control. They also found that the use of perben-ice (propyl paraben 0.1%) for preserving fish, instead of ordinary ice, resulted in the extension of shelf life of fish by 18 days over that of control. This experiment was, however, carried out on a laboratory scale using 2-3 kg fish per batch. It can be inferred from these studies that the extension of shelf life obtained under laboratory conditions could not, however, be achieved under semi-commercial trials. While the laboratory studies gave an extended shelf life of 18 days, as compared to control when perben-ice was used, the same results could not be achieved in the present study when done on a semi-commercial scale, using the same fish and same concentration of propyl paraben to ice. This is probably true in most cases, where transfer of results from the laboratory to the field are involved. In this particular case, the foremost important factor responsible for reduced shelf life appear to be inadequate contact of ice with the fish, due to bulk storage. The other important factors appear to be physical damage to fish while handling, inadequate cooling, pressure exerted on fish (all due to bulk storage), the effects of season and fishing grounds and various biological factors involved in the growth and physiological functions of microorganisms.

Extension of shelf life by ten days as obtained in this study for croaker, catfish and tresh fish appears to be significant from the commercial point of view. Propyl paraben is active against Gram positive and Gram negative bacteria (Shirelkar, 1971; Anand and Setty, 1981; Singh, 1978), and being effective over a wide range of pH (6.5-9.5). It is easily metabolizable to the mammalian system and non-toxic (Shirelkar, 1971) has a high potential for commercial exploitation. Esters of p-hydroxybenzoic acid have been allowed as preservatives for various food items, like beverages, baked goods, salads, dried fruits and vegetables and pickles (Bauerfeind and Pinkert, 1970) and in many countries. However, perben esters have not been considered for fish and meat so far by many countries, since according to earlier reports these perben esters are not effective against gram negative bacteria, which are mainly responsible for spoilage of meat and fish.

4. REFERENCES

Anand, C.P., M.F. Sc. Thesis, University of Agricultural Sciences, Bangalore
1976

- Anand, C.P. and T.M.R. Satty, Studies on the chemical control of psychrophilic bacterial spoilage of fish. 3. The affect of chemical preservatives on the growth of psychrophilic bacteria isolated from marine fish. Fish.Techmol.Soc.Fish.Techmol., Ernakulam, 18:47-53 1981
- APHA (American Public Health Association), Compendium of methods for the microbiological examination of foods. Edited by M.L. Speck. Washington, D.C., APHA 1976
- Bauerfeind, J.C. and D.M. Pinkert, Food processing with added ascorbic acid. Adv.Food Res., 1970 18:220-315
- Baatty, S.A. and M.E. Gibbons, The measurement of spoilage in fish. J.Biol.Board Can., 3:77-91 1937
- Castell, C.N. and R.E. Triggs, Spoilage of haddock in the trawlers at sea: the measurement of 1955 spoilage and standards of quality. J.Fish.Res.Board Can., 12(3):329-41
- Connell, J.J., Control of fish quality. Farnham, Surrey, England, Fishing News (Books) Ltd. 1975
- Farber, L., Freshness tests. In Fish as food, edited by G. Borgstrom. New York, Academic Press, 1965 vol. 4:65-126
- Glassman, G. and F. Rochwarger, Ein Beitrag Zum Nachweis der beginnenden Fleischfaulnis Und ubar ihre Bestimmungsmethoden Von in Salzform gebundenen Ammoniak in Fleisch. Z.UnTERS, Lebensmitt., 58:585-92 1929
- Kimura, K. and S. Kiamukura, Detection of the onset of decomposition in fish meat as shown by the content of ammonia. Proc.Pac.Sci.Congr., 5:3709 1934
- Shewan, J.M., The bacteriology of fresh and spoiling fish and some related chemical changes. In 1962 Racant advances in food science, edited by J. Hawthorn and J.M. Leitch. London, Butterworths
- Shiralkar, N.D., P-Hydroxybenzoates as food preservatives. Ph.D. Thesis, University of Bombay, 1971 India
- Singh, K., Studies on the use of propyl paraben for the preservation of marine fish at low temperatures (0-5°C). M.F. Sci. Thesis, University of Agricultural Sciences, Bangalore, 1978 Karnakata, India
- Sigurdsson, G.J., Comparison of chemical tests of the quality of fish. Anal.Chem., 19:892-902 1955
- Shimizu, M., The changes in nitrogen compounds during fish spoilage. J.Agric.Chem.Soc.Japan, 1925 1:730-9
- Tenikawa, E., Studies on measuring freshness of fish and shellfish meat. J.Soc.Ind.Fish., Japen 1935 3:267-96 (in Japanese)
- Tetcher, F.S. and D.S. Clerk, Microorganisms in food; their significance and methods of enumeration. Toronto, University of Toronto Press 1968
- Tillmans, J. and R. Otto, Ueber den Nachweis der beginnenden Fischfaulnis. Z.UnTERS,Nahr, Genussmitt., 47:25-37 1924
- Yemure, Y., The putrefaction degree and the pH value of fish muscle. Bull.Jap.Soc.Sci.Fish., 1933 2:118-20

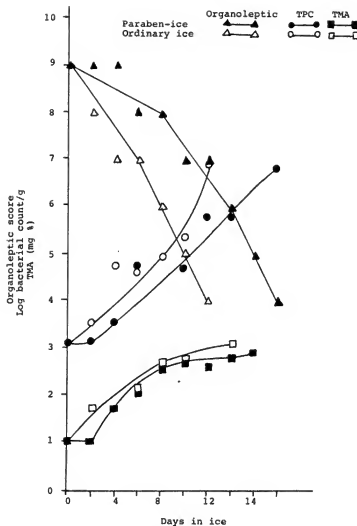


Figure 1 Shelf life of threadfin bream (*Nemipterus japonicus*) during storage in ordinary ice and paraben-ice (batch I)

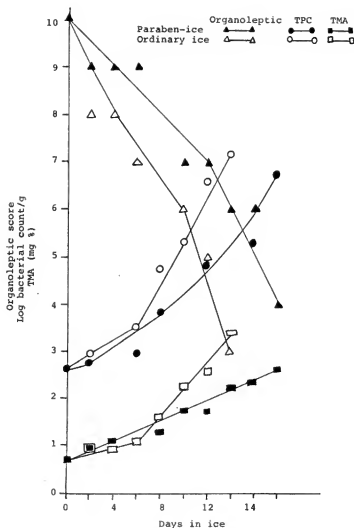


Figure 2 Shelf life of threadfin bream (*Hemipterus japonicus*) during storage in ordinary ice and paraben-ice (batch II)

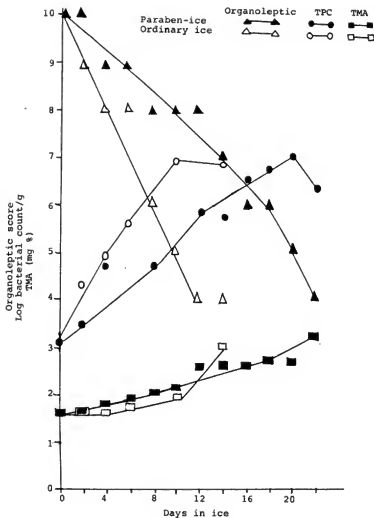


Figure 3 Shelf life of croaker (*Johneops* sp.) during storage in ordinary ice and paraben-ice

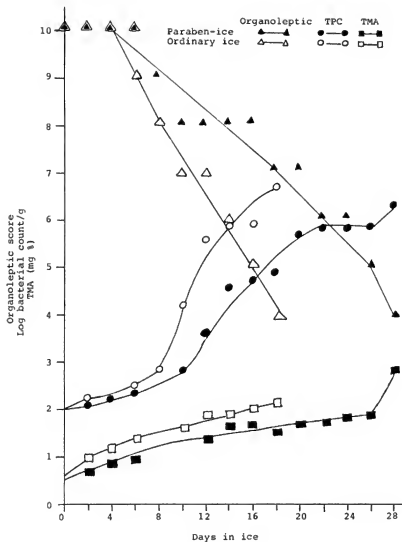


Figure 4 Shelf life of catfish during storage in ordinary ice and paraben-ice

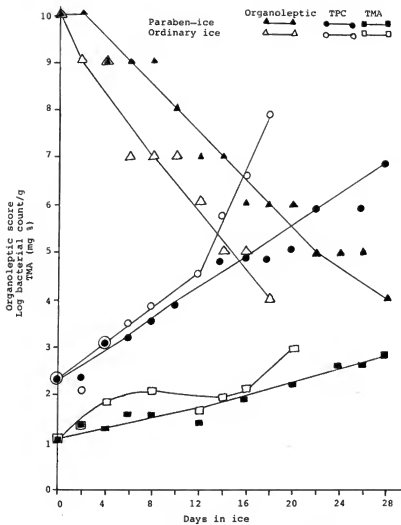


Figure 5 Shelf life of trash fish (mixed species) during storage in ordinary ice and paraben-ice

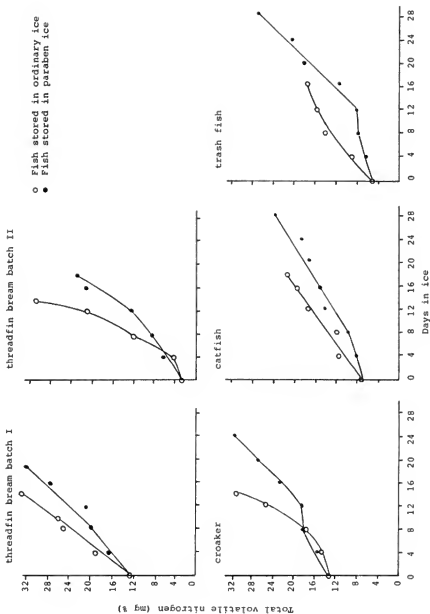


Figure 6 Changes in total volatile nitrogen levels during storage

COMPARATIVE STUDY OF FISH BACTERIA FROM TROPICAL
AND COLD/TEMPERATE MARINE WATERS

by

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ABSTRACT

The generic distribution of bacteria of some freshly caught tropical marine fishes (Indian mackerel, oil sardine, croaker and pink perch) was found to be dominated by *Flavobacterium*, *Pseudomonas*, *Aeromonas*, *Moraxella*, *Alcaligenes* and *Vibrio* species among the Gram negative flora. Among Gram positives, *Micrococcus* spp. were found in higher numbers followed by *Corynebacterium* and *Bacillus* species. These tropical bacterial isolates, and the cold/temperate marine fish bacteria, were compared for their growth and biochemical activities. The cold/temperate bacterial genera grew better at lower temperatures (2 and 8°C), than the tropical isolates and also showed comparatively better biochemical activities. Only the *Moraxella* and *Corynebacterium* groups from both tropical and cold/temperate waters, could grow well even at 37 and 42°C.

1. INTRODUCTION

A large number of systematic studies on the cold/temperate fish bacteria have been carried out and an abundant volume of data are available as to their morphological, physiological and biochemical characteristics. However, very few such studies have been carried out on tropical fish bacteria in India and other countries. As such there is a need for a detailed study of these tropical fish bacteria. Wood (1967), Horsley (1977) and Lima dos Santos (1981) have reviewed the work done on the tropical and cold/temperate marine fish bacteria and have suggested that a comprehensive study is essential and that only after obtaining enough data on the tropical fish bacteria it would be possible to draw useful and meaningful conclusions. The present work is an attempt to understand the bacteriology of four important tropical marine fish and to compare the growth and biochemical characteristics with those of cold/temperate marine fish bacteria.

2. MATERIALS AND METHODS

The fish species Indian mackerel (*Rastrelliger kanagurta*), Indian oil sardine (*Sardinella longiceps*), pink perch (*Neimipterus japonicus*) and croaker (*Johannes* spp.) caught off Mangalore coast were sampled on board the fishing vessel. The samples were collected in sterile polythene bags and brought to the laboratory in insulated boxes with crushed ice and were used immediately for bacteriological analysis.

All the diluents, media and broths were prepared in the laboratory according to the methods of the APHA (1976).

The four fish samples taken in this study were tested for total aerobic plate counts, using whole fish homogenate and drop plate technique. Plates were incubated $28 \pm 2^\circ\text{C}$ for 48 h and the counts recorded. The 459 bacterial isolates obtained from four samples were identified up to their generic level by making use of the scheme suggested by Shewan, Hobbs and Hodgkiss (1960) and Lechevallier, Seider and Evans (1980).

Out of the 459 isolates identified up to their generic level, 97 isolates, representing different bacterial genera, were selected for the study reported in this paper and are shown in Table 1. Twenty-six bacterial cultures isolated from cold/temperate marine fish were obtained from the Torry Research Station, Aberdeen (UK), the list of which is given in Table 2.

For the purpose of growth studies, all the 123 isolates (i.e., both tropical and cold/temperate) included in the study were inoculated into sterile nutrient broths and incubated at $28 \pm 2^\circ\text{C}$ for 24 h. After incubation, one loopful of each culture was transferred to each of the five tubes of 10 ml sterile nutrient broth in all the cases, thus getting five sets of cultures. One set each from these was then incubated at $2 \pm 1^\circ\text{C}$, $8 \pm 1^\circ\text{C}$, $28 \pm 2^\circ\text{C}$, $37 \pm 1^\circ\text{C}$ and $42 \pm 1^\circ\text{C}$ for varying periods. In each case the growth (turbidity) was measured in terms of optical density using Spectronic 21 colourimeter at regular intervals.

Some of the biochemical characteristics of both the cold/temperate and tropical marine fish bacteria studied were gelatin hydrolysis, starch hydrolysis, H_2S production, citrate utilization, nitrate reduction and action on sugars.

3. RESULTS AND DISCUSSION

In the present study, the total bacterial counts, immediately after catch, for the two pelagic fishes, namely Indian mackerel and oil sardine, were found to be 3.05×10^7 and 3.75×10^7 /g (whole fish) respectively (Table 3). Jedhev and Mager (1970) reported total bacterial counts of 2.1×10^7 /g of whole fish for Indian mackerel; Surendran and Gopekumar (1982) reported 10^7 - 10^8 /cm of skin, 10^7 - 10^8 /g of gills and 10^7 - 10^8 /g of guts for Indian mackerel. In the case of oil sardine also, the reported total bacterial counts are 10^7 - 10^8 /cm² of skin (Karthikeyani and Mahadeva Iyer, 1971); 10^7 - 10^8 /cm² of skin, 10^7 - 10^8 /g of gills and 10^7 - 10^8 /g of guts (Surendran and Gopekumar, 1982). The total bacterial counts in the case of the two demersal fishes, croaker and pink perch, were found to be 3.55×10^7 and 3.4×10^7 /g of whole fish respectively (Table 3). Anand (1976) has reported total bacterial counts for freshly caught croaker and pink perch to be of the order of 0.3×10^7 and 1.1×10^7 /g of whole fish respectively.

Reports on total bacterial counts for tropical marine fish species are rather scarce, as compared to the data available for the cold/temperate water fish species and there are wide variations in the reported values ranging from 10^7 - 10^8 /cm² of skin, 10^7 - 10^8 /g of gills and 10^7 - 10^8 /g of guts. It may be relevant to point out here that in most of the published work on tropical fish bacteriology, the information on the catching grounds, condition of the fish at the time of sampling - that is to say, whether fresh immediately after catch or iced at the landing centre, or if the fish has been obtained from the local market and its past history - are lacking. Also, lack of uniformity in sampling and plating technique adopted by various workers will introduce a lot of difficulties in comparing such data with those obtained for cold/temperate water fish species, where systematic analysis has been done. Because of this anomaly, Shewen (1977), while trying to compare total bacterial load on warm and cold water fish species, was inclined to conclude that fish from warm waters frequently carry greater number of bacteria than cold/temperate water fish species. Lima dos Santos (1981), after comparing most of the available data on warm-water fish species, suggested that the size of the bacterial population on the skin of the marine fish is within the limits quoted by Shewen and Hobbs (1967) for fresh cold/temperate marine fish species (10^7 - 10^8 /cm²). The results obtained in the present study compare very well with the reported values for these fishes by other workers.

3.1 Distribution Pattern of Bacterial Genera in Fish

The results of this study, based on 459 isolates (Table 4), show the dominance of *Flavobacterium* spp. (12.2%), followed by *Pseudomonas* (10.82%), *Aerobacter* (8.71%), *Moraxella* (7.84%), *Alcaligenes* (4.13%) and *Vibrio* (1.3%) species among the Gram negative flora. Among Gram positives, *Micrococcus* spp. (27.22%) were found in greater percentage, followed by *Corynebacterium* spp. (18.3%) and *Bacillus* spp. (9.15%). The generic distribution of bacteria for pelagic fishes also showed higher percentages of *Flavobacterium* spp. (13.44%), followed by *Pseudomonas* spp. (12.59%), *Moraxella* spp. (7.99%) and *Alcaligenes* spp. (4.85%) among the Gram negative flora. Similarly for demersal fishes also, the *Flavobacterium* group (10.94%) dominated, followed by *Pseudomonas* (9.13%), *Aerobacter* (9.68%), *Moraxella* (5.98%), *Alcaligenes* (3.2%) and *Vibrio* species (2.75%). In the case of both pelagic and demersal fishes, the Gram positive bacterial flora was dominated by *Micrococcus* spp., followed by *Corynebacterium* spp. and *Bacillus* spp., except that the percentage of *Bacillus* was found to be more in demersal fish (13.17%), than in pelagic fish (5.32%). The close proximity of the demersal fish to the bottom sediments of the ocean, which is known to carry more of Gram positive bacteria, probably explains the higher percentages obtained in this study for demersal fishes. Surendran and Gopekumar (1981), while analysing 82 cultures isolated from oil sardines, found *Vibrio* spp. (26%) to dominate followed by *Aerobacter* (24%), *Pseudomonas* (16%), *Moraxella* (8%) and *Flavobacterium* species (5%), whereas in mackerel, they reported the distribution of 64 cultures to be dominated by *Vibrio* (42%), followed by *Aerobacter* (20%), *Pseudomonas* (16%), *Moraxella* (10%) and *Flavobacterium* species (3%). The same authors in 1982 found almost the same distribution pattern of bacterial flora for oil sardine and mackerel, with only marginal changes in the percentages of various bacterial genera. The fishes caught off Mangalore coast appear to show dominance of *Flavobacterium* spp. over other Gram negatives (Anand, 1976; Singh, 1978), whereas the fishes from Cochin waters appear to show the dominance of *Vibrio* spp. (Surendran and Gopekumar, 1981, 1982). The general composition of the Gram negative bacterial flora of the tropical marine fishes, to consist of *Aerobacter* (*Moraxella*, *Aerobacter* and *Alcaligenes* species), *Pseudomonas*, *Flavobacterium* and *Vibrio* species reported recently by Shewen (1977) and Lima dos Santos (1981) agrees well with the findings of this study. However, there seems to be significant differences between the percentages of total Gram negatives and total Gram positives reported earlier and results obtained in the present study, where there is slight dominance of Gram positives over Gram negatives. The numerical relationship of one group to another may tend to vary with the season, as well as with the catching grounds (Lamprecht, 1961).

3.2 Growth Studies

All the bacterial isolates from tropical and cold water fish included in the study showed growth at 2°C, except that the rate of growth of tropical isolates was rather slow and required a minimum of 8-10 days to show visible growth, as against 6 days for bacterial isolates from cold water fish. At 8°C, majority of the isolates from cold water fish showed a far better growth rate (0.10 to 0.23, O.D. in 8 days) as compared to a poor growth rate for the isolates from tropical fish (0.04 to 0.10, O.D. in 8 days) (Figures 1 and 2). Since *Pseudomonas* spp. are the major spoilage bacteria, both in tropical and cold water, their growth rate at 8°C is of greater importance than their growth rate at higher temperatures. Generally, fish is stored in ice and the temperature of which under commercial conditions is likely to be in the range of 5-8°C and hence those species of bacteria that can grow fast in this range of temperature are the ones that dominate the spoilage flora of fish. The fact that all the *Pseudomonas* species (including *Alteromonas*) both from tropical and cold water fish show better growth at 8°C than other groups of bacteria appears to be a very significant common factor between these two groups of bacteria (Figures 1 and 2). The *Vibrio* spp. and *Bacillus* spp. from tropical fish, which come close to *Pseudomonas* spp. in their growth rate at 8°C, also appear to be important as far as spoilage of tropical fish is concerned. Moreover, recent reports on the tropical fish bacteriology indicate greater percentages of *Vibrio* spp. among the flora of fresh fish (Surendran and Gopakumar, 1981, 1982). The *Bacillus* spp. although not found to be predominant among pelagic fishes, appear to be fairly significant among the demersal fishes.

The fact that tropical fish bacteria show slower growth rate than those of cold water species at 8°C probably answers the slightly increased shelf life generally noticed in tropical fish stored in ice, as compared to the shelf life of ice stored fish from cold/temperate marine waters.

Most of the bacterial cultures from tropical fish showed much better growth rates than those from cold water fish at 28°C (Figures 3, 4 and 5). This reversal in the pattern of growth rate at higher temperatures may be of particular significance to Indian processors, wherever there is delay in icing and during processing operations and transport of iced fish over long distances. This study also indicates that the cold water bacteria, although capable of growth at 28°C, appear to be more sensitive, in general, to higher temperatures. This fact becomes even more evident at 37 and 42°C for the majority of the cold water as well as tropical bacterial species, except for *Moraxella* and *Corynebacterium* groups, which could grow well even at 37 and 42°C (Figure 6).

Comparing the growth rate of various bacterial genera at 8 and 28°C and putting them in descending order, the pattern appeared to be the same for both tropical and cold/temperate fish bacteria. The order was found to be *Pseudomonas*, *Flavobacterium*, *Moraxella*, *Acinetobacter* and *Corynebacterium* species for 8°C and for 28°C the order was *Acinetobacter*, *Corynebacterium*, *Moraxella*, *Pseudomonas* and *Flavobacterium* species (excluding *Bacillus*, *Vibrio*, *Alcaligenes* and *Micromonospora* species from the list of tropical fish bacteria, since the corresponding groups from cold/temperate fish bacteria could not be obtained for this study). *Pseudomonas* and *Flavobacterium* groups which were at the top of the list at 8°C came down to the bottom at 28°C, indicating the effect of temperature on the most important groups of fish spoilers.

3.3 Biochemical Characteristics

Excluding the bacterial genera, namely *Bacillus*, *Micromonospora*, *Alcaligenes* and *Vibrio* species, the cold-water species which were not included in the present study, and comparing the remaining genera, a striking similarity in the biochemical abilities of isolates from tropical and temperate marine fish was evident from the results obtained in this study (Figures 6, 7, 8, 9 and 10).

Among the active spoilers, *Pseudomonas* species from both tropical and temperate fish were found to be outstanding, although 100% of the cold-water species had ability to hydrolyse gelatin as against 66-100% for tropical isolates, except *Pseudomonas* spp. group-1 (22%). Except citrate utilization, which was negative for both tropical and cold water species of *Pseudomonas*, the latter group showed slightly better capability than the former for most other biochemical activities. However, it should be mentioned here that there was only one standard culture for each group of *Pseudomonas* among the cold water species; as such, the above generalization should be viewed keeping this limitation in mind.

Among other genera, the *Moraxella* spp. from cold waters showed slightly better ability than the tropical species in most of the biochemical characteristics. The same was true in the case of *Acinetobacter*, *Corynebacterium* and *Flavobacterium* species. The bacterial isolates from cold/temperate water fish, in general, were found to be more active than the tropical isolates in their biochemical characteristics.

4. REFERENCES

- Anand, C.P., M.F. Sc. Thesis, University of Agricultural Sciences, Bangalore, Karnataka
1976

- APHA (American Public Health Association), Compendium of methods for the microbiological examination of foods, edited by M.L. Spack. New York, APHA
- Horsley, R.W., A review of the bacterial flora of teleosts and elasmobranchs, including methods for its analysis. J.Fish Biol., 10(6):529-33
- Jadhav, M.G. and N.G. Magar, Preservation of fish by freezing and glazing. 1. Bacteriology of fresh, frozen and glazed fish. Fish.Technol., Soc.Fish.Technol., Ernakulam, 7(1):86-90
- Karthiayeni, T.C. and K. Mahadeva Iyer, Seasonal variations of bacterial flora of fresh oil sardines (*Sardinella longirostris*). Fish.Technol., Soc.Fish.Technol., Ernakulam, 8(1):69-7
- Lamprecht, E., 15th Annual Report FRI, South Africa, pp. 14-6
1961
- Lechavallier, M.W., R.J. Seider and T.M. Evans, Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Appl.Environ.Microbiol., 40(5):922-30
- Lima dos Santos, C.A.M., The storage of tropical fish in ice - a review. Trop.Sci., 23:97-127
1981
- Shewan, J.M., The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In handling, processing and marketing of tropical fish, London, Tropical Products Institute, pp. 51-66
- Shewan, J.M. and C. Hobbs, In Progress in Industrial Microbiology, edited by A.J.D. Mockenhull. 1967 London, Heywood Books, 6:171-208
- Shewan, J.M., C. Hobbs and W. Hodgkiss, J.Appl.Bacteriol., 23(3):463-8
1960
- Singh, K., M.F.Sc.Thesis, University of Agricultural Sciences, Bangalore, Karnataka
1978
- Surendran, P.K. and K. Gopakumar, Selection of bacterial flora in the chlortetracycline treated oil sardine (*Sardinella longirostris*), Indian mackerel (*Rastrelliger kanagurta*) and prawn (*Metapenaeus dohrnii*) during ice storage. Fish.Technol., Soc.Fish.Technol., Ernakulam, 18(2):133-41
- _____, The bacteriology of oil sardine (*Sardinella longirostris*) and mackerel (*Rastrelliger kanagurta*) caught in tropical waters off Cochin. 1. Quantitative aspects. Fish.Technol., Soc.Fish.Technol., Ernakulam, 19(2):89-96
1982
- Wood, E.J.F., Fish spoilage. In Microbiology of oceans and estuaries, Amsterdam, Elsevier Publishing Co., Elsevier oceanography series, 3:242
1967

Table 1

List of bacterial cultures from tropical fish used in the study

Name of the culture	Number of cultures	Remarks
<u>Acinetobacter</u> spp.	8	All these bacterial cultures were isolated from Indian mackerel, Indian oil sardine, croaker, pink perch caught off Mangalore coast
<u>Alcaligenes</u> spp.	8	
<u>Bacillus</u> spp.	6	
<u>Corynebacterium</u> spp.	9	
<u>Flavobacterium</u> spp.	9	
<u>Micrococcus</u> spp.	7	
<u>Moraxella</u> spp.	9	
<u>Pseudomonas</u> group-I	9	
<u>Pseudomonas</u> group-II	9	
<u>Pseudomonas</u> group-III	9	
<u>Pseudomonas</u> group-IV	9	
<u>Vibrio</u> spp.	5	

Table 2

List of bacterial cultures from temperate fish used in the study (obtained from Torry Research Station, Aberdeen, UK)

Name of the culture	Number of isolates	Remarks
<u>Corynebacterium</u> spp.	4	Bacterial cultures from cod and whiting fishes (isolated by T.M.R. Setty during his visit to Torry Research Station in 1978-79. Isolated on plate count agar at a primary isolation temperature of 25-28°C.
<u>Moraxella</u> spp.	5	
<u>Pseudomonas</u> spp.	5	
<u>Acinetobacter</u> spp.	3	
<u>Flavobacterium</u> spp.	2	
<u>Pseudomonas</u> fluorescence	1	Standard cultures from the culture collection of Torry Research Station, Aberdeen, U.K.
<u>Alteromonas</u> spp.	1	
<u>Moraxella</u> -like spp.	1	
<u>Pseudomonas</u> fragi	1	
<u>Pseudomonas</u> putida	1	
<u>Vibrio</u> anguillarum	1	
<u>Alteromonas</u> putrefaciens	1	

Table 3

Total aerobic plate counts of bacteria for tropical marine fish species

Sample No.	Fish species	Log bacterial count/g
1	Indian mackerel (<u>Rastrelliger kanagurta</u>)	3.05×10^5
2	Indian oil sardine (<u>Sardinella longiceps</u>)	3.75×10^5
3	Croaker (<u>Johneops</u> spp.)	3.55×10^4
4	Pink perch (<u>Nemipterus japonicus</u>)	3.4×10^4

Table 4

Percentage generic distribution/composition of bacterial flora isolated from tropical fish species

Name of the genus	Distribution for pelagic fish	Distribution for demersal fish	Overall generic distribution of isolates
No. of isolates studied	241	218	459
Temperature of incubation (°C)	28 ± 2	28 ± 2	28 ± 2
<u>Acinetobacter</u> spp.	7.99	9.68	8.71
<u>Alcaligenes</u> spp.	4.85	3.20	4.13
<u>Flavobacterium</u> spp.	13.44	10.94	12.20
<u>Moraxella</u> spp.	9.20	5.98	7.84
Total <u>Pseudomonas</u> spp.	12.58	9.13	10.87
<u>Pseudomonas</u> spp. group-I	2.42	1.39	1.96
<u>Pseudomonas</u> spp. group-II	4.22	2.78	3.48
<u>Pseudomonas</u> spp. group-III	3.87	2.73	3.26
<u>Pseudomonas</u> spp. group-IV	2.07	2.23	2.17
<u>Vibrio</u> spp.	-	2.75	1.30
Total Gram negatives	48.06	41.68	45.05
<u>Micrococcus</u> spp.	27.23	27.21	27.22
<u>Corynebacterium</u> spp.	19.38	17.94	18.30
<u>Bacillus</u> spp.	5.32	13.17	9.15
Total Gram positives	51.93	58.32	54.67

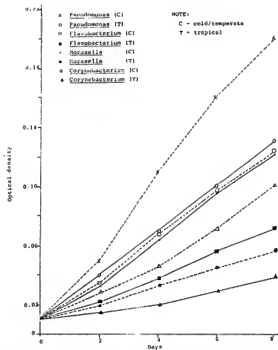


Figure 1 Growth of bacterial isolates from tropical and cold water marine fish at 8°C

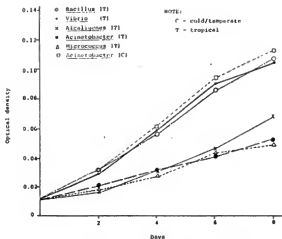


Figure 2 Growth of bacterial isolates from tropical and cold water marine fish at 8°C

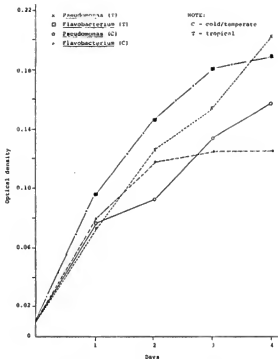


Figure 3 Growth of bacterial isolates from tropical and cold water marine fish at 28°C

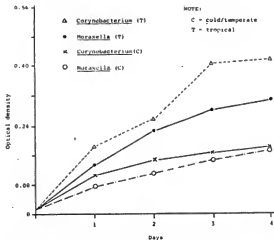


Figure 4 Growth of bacterial isolates from tropical and cold water marine fish at 28°C

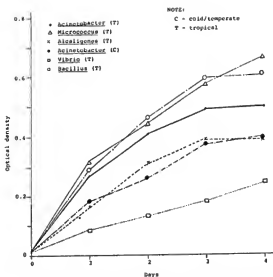


Figure 5 Growth of bacterial isolates from tropical and cold water marine fish at 28 °C

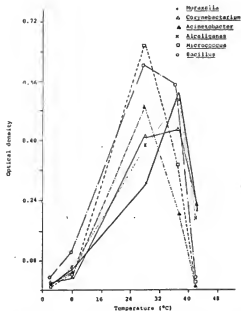


Figure 6 Growth of bacterial isolates from tropical marine fish at different temperatures

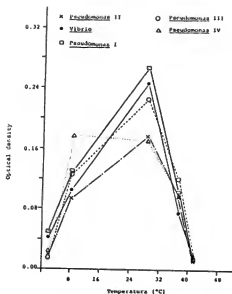


Figure 7 Growth of bacterial isolates from tropical marine fish at different temperatures

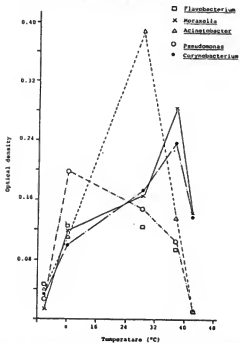


Figure 8 Growth of bacterial isolates from cold/temperate marine fish at different temperatures

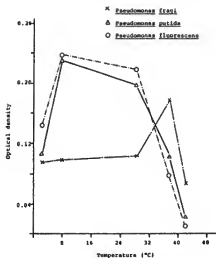


Figure 9 Growth of bacterial isolates from cold/temperate marine fish at different temperatures

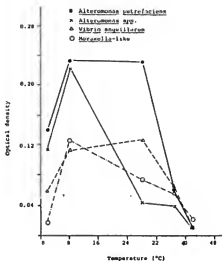


Figure 10 Growth of bacterial isolates from cold/temperate marine fish at different temperatures

HANDLING OF SIX SPECIES OF FRESH FISH OF BANGLADESH

by

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ABSTRACT

Studies on seasonal variation in chemical composition and some aspects of quality changes during handling and short-term preservation of the following six species of fish were undertaken: (1) *Puntius stigma*, (2) *Glossogobius giuris*, (3) *Tilapia nilotica*, (4) *Cyprinus carpio*, (5) *Macrobrachium rosenbergii* and (6) *Stromateus sinensis*. Proximate composition varied with species, sex, maturity, different anatomical portions and also different seasons. In winter, higher fat and lower moisture contents were observed in all species. Juveniles were found to contain more protein but less fat than adults.

The fish were kept at different storage temperatures and their spoilage pattern and the shelf life were studied by subjective and objective parameters. The spoilage rates were found to increase with the increase of storage temperature for all the six varieties.

The effects of containers on the keeping quality of fresh fish with ice were also studied. Bamboo baskets and wooden boxes, insulated with hoglamat (prepared from the spongy leaves of a local plant), were found to be most efficient.

1. INTRODUCTION

Bangladesh is a riverine country with vast fisheries potential. There is an area of 1.58 million hectares of inland water and 283 400 ha of paddy fields which are submerged for 4-6 months. Bangladesh produces a total of 0.64 million tons of fish of which 0.52 million tons are from inland waters and 0.12 million tons of marine fish. The production is too low to meet the country's demand. The immediate problem is to increase production and also to prevent post-harvest losses. Most of the inland water catch is consumed fresh, but due to the absence of necessary infrastructure for transportation, handling, marketing and lack of proper facilities at the landing centres for ice, fish boxes, etc., substantial quantities of fish spoil and become unfit for human consumption. Moreover, returns to the fishermen are reduced. Very little technological attention has so far been paid for its proper handling, preservation and to characterize the nature of spoilage. Species like *Puntius stigma* (puti), *Glossogobius giuris* (Bele), *Tilapia nilotica*, *Cyprinus carpio*, *Macrobrachium rosenbergii* and *Stromateus sinensis* were selected for this investigation, which represent common commercial varieties.

2. EXPERIMENTAL

2.1 Raw Materials

2.1.1 Source of specimen

Puntius stigma (puti) and *Glossogobius giuris* (Bele) were collected directly from fishermen in fresh condition. The giant prawn *Macrobrachium rosenbergii* of family Palaemonidae (local name: golda or shala or nowla chingri) were collected from the landing centre of Maghma ghat in fresh condition. *Stromateus sinensis* (local name: Rupchanda, English name: Chinese pomfret) were collected from the wholesale fish market of Bangladesh Fisheries Development Corporation (BFDC) at Cox's Bazar. These were then packed into cartons in polyethylene bags with ice, and were brought to Dhaka by air.

Tilapia nilotica and *Cyprinus carpio* were collected from a fish seed-multiplication farm at Tongi, Dhaka.

The fish for summer analysis were collected in the latter half of July and that for winter analysis in the latter half of December. Females bearing eggs were collected in the months May-June.

2.1.2 Identification and sampling

As soon as the fish reached the laboratory, they were separated according to different species, sex, size, for different experiments.

2.2 Proximate Composition

Moisture, protein, ash and fat content of the six varieties of samples were determined by the method of AOAC (1975).

2.3 Effect of Using Water Weeds on Spoilage Changes in Sunlight and in Shade

About 80% of the inland fish are caught in rural areas of Bangladesh. Due to nonavailability of ice in the remote areas, the fishermen generally use aquatic weeds to cover fish to retard spoilage and thus protect the catch as a short-term measure at landing centres and during transportation to the urban market. There is no report available on the effectiveness of this traditional technique in reducing the spoilage rate of fish. The present investigation was undertaken to measure the effectiveness of this traditional technique for the following fishes (head on, head off): *Puntius stigma*, *Glossogobius giuris*, *Tilapia nilotica*, *Cyprinus carpio* and *Macrobrachium rosenbergii*.

The five species were divided into the following four batches, each batch containing about 50 fish which were kept in bamboo baskets with or without water weeds, in sunlight and in shade:

- Batch 1 - in sunlight without water weeds
- Batch 2 - in sunlight with water weeds
- Batch 3 - in shade without water weeds
- Batch 4 - in shade with water weeds

At night all batches were kept in a room (at +15°C). Occasional sprinkling of water was done for all experiments where ice was not used for a triple affect: (1) to prevent dehydration, (2) to wash out the surface bacteria, (3) to release latent heat. Fish were turned to ensure uniformity of temperature. The experiment was carried out in summer. The samples were collected at random at an interval of 0 h, 2 h, 4 h, 8 h, 10 h, 21 h and 23 h of storage to assess the quality. In summer, the room temperature was 29°-30°C and outside temperature was 33°-34°C.

2.4 Effect of Different Storage Temperature on Spoilage Changes

Fish being a highly perishable commodity requires proper care during handling, transportation, distribution and marketing. In Bangladesh fish are normally transported under different conditions. Changes in temperature at different stages of transportation result in the deterioration of the quality at different rates. It was therefore felt necessary to study the effect of different temperatures during storage which is likely to provide useful information for commercial transportation and preservation.

Puntius stigma, *Glossogobius giuris*, *Tilapia nilotica*, *Cyprinus carpio* and *Stromateus sinensis* were used for this investigation.

About 150 fish of each species (except pomfret) were divided into four batches and were subjected to different temperatures for different lengths of time until the fish became unacceptable. In the case of pomfret only 24 fish were divided and composite samples were taken for investigation. Fish were kept in batches as follows:

- Batch 1 - superchilling temperature, -2°C
- Batch 2 - in ice, 0°C
- Batch 3 - refrigerated temperature, +7°C ± 1°C
- Batch 4 - cold-room temperature +15°C ± 2°C

The fish were removed at random from each batch at different intervals of 0, 1, 2, 3, 7, 10, 13, 16 and 20 days of storage to assess the quality.

2.5 Studies on the Effect of Size on the Rate of Spoilage

Only *Puntius stigma* and *Glossogobius giuris* were taken for this investigation. Two sizes of *Puntius stigma*, large (about 9-10 cm) and small (about 4-5 cm) and three sizes of *Glossogobius giuris*, large (about 14-19 cm), medium (about 9-13 cm) and small (about 5-8 cm) were taken for the

experiment. About 100 *Puntius stigma* and *Glossogobius giuris* were divided according to size. The samples were stored at room temperature (29°-30°C). The fish were kept in bamboo baskets without ice. Samples were collected at random at an interval of 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h and 24 h of storage to assess the quality.

2.6 Studies on the Effect of Species on Spoilage Changes

Puntius stigma, mixed with *Puntius sarana* and *Puntius ticto*, as well as *Glossogobius giuris* mixed with *Gobius sadanandio* and *Gobius melanogonius*, were used in this experiment. The experiment was carried out at room temperature (29°-30°C). Each species was kept separately in a bamboo basket without any covering. At intervals of 0 h, 3 h, 6 h, 9 h, 12 h, 14 h and 24 h of storage, samples were collected at random to assess the quality.

2.7 Studies on the Effect of Container on Spoilage Changes During Ice Storage

Cooling with ice can slow down the spoilage process and prolong the shelf life of fish. In Bangladesh, where ice is available, fish is packed in different types of containers such as metal boxes, bamboo baskets, wooden boxes, etc. The insulating properties of the containers are among the factors which influence the rate of melting of ice, which in turn contributes to the changes in quality of fish during ice storage.

For the present experiment, *Puntius stigma*, *Glossogobius giuris*, *Tilapia nilotica* and *Macrobrachium rosenbergii* (head on, head off) were taken. Different types of containers with ice were used to evaluate the storage life in ice. The containers for different fish were as follows:

Puntius stigma

- (a) Fish with and without polyethylene bag kept in wooden box with ice.
- (b) Fish with and without polyethylene bag kept in bamboo baskets with ice.
- (c) Fish with and without polyethylene bag kept in metallic container with ice.

Glossogobius giuris

- (a) Fish wrapped with hoglamat and without wrapping kept in wooden box with ice.
- (b) Fish wrapped with hoglamat and without wrapping kept in bamboo basket with ice.

Tilapia nilotica and *Cyprinus carpio*

- (a) Fish in polyethylene bag kept in wooden box and bamboo basket with ice.
- (b) Fish wrapped with hoglamat kept in wooden box and bamboo basket with ice.
- (c) Fish in metal container with ice.

Macrobrachium rosenbergii (head on, head off)

- (e) Fish with and without polyethylene bag kept in wooden box with ice.
- (h) Fish with and without polyethylene bag kept in bamboo basket with ice.

About 300-400 fish of the above species were divided between the different containers. The fish were placed over a thick layer of crushed ice, above which another layer of crushed ice was spread. The ratio of ice and fish was 4:1. The container with ice was kept at room temperature (about 29°C). About 4-5 fish were removed at random from each group at intervals of 0, 1, 3, 6, 10, 14 and 15 days of storage to assess the quality.

2.7.1 Assessment of quality

The quality of fish was determined by sensory judgement and by measuring spoilage changes by chemical methods.

2.7.1.1 Organoleptic score: The score sheets used followed the score sheet developed at the Torry Research Station, UK. Scoring was out of 100 for the different quality aspects of fresh fish.

(a) <u>Acceptability</u>			(f) <u>Odour</u>		
Highly acceptable	HA	10	Fresh fishy odour		10
Acceptable	A	8	Loss of fishy odour/sweet		8
Moderately acceptable	MA	6	Slight spoils odour		7
Just acceptable	JA	5	Spoilage odour		5
Just unacceptable	JUA	4	Slight off odour		3
Unacceptable	UA	3	Off odour		2
More unacceptable	MUA	2	Extremely off odour		0
Extremely unacceptable	EUA	0			
(b) <u>Eye</u>			(g) <u>Texture</u>		
Bright clear, and full		10	Firm and elastic		10
Slight dull		9	Rigor stage (firm)		9
Moderately dull		8	Just post rigor stage		7
Dull		7	Slightly soft		5
Slightly sunken		6	Moderately soft		3
Moderately sunken		4	Just retaining impression imprints		1
More sunken		2	Very soft and loose		0
Completely sunken		0			
(c) <u>Pupil</u>			(h) <u>Flesh Condition</u>		
Bright and transparent		10	Translucent		10
Slight dull and milky		8	Loss of translucency		8
Slight milky white		7	Slightly dull		6
Watery colour/whitish		6	Dull		5
Milky white		4	Dull red		4
Pale/white		2	Reddish loose/light wax		2
Extremely pale/more white		0	Reddish/loose and separable from bones		0
(d) <u>Gills</u>			(i) <u>Viscera</u>		
Bright red		10	Fresh condition		10
Slight dull red		9	Loss of fresh colour		9
Slight pale		7	Slightly digested		8
Moderately pale		6	Moderately digested		6
Pale/whitish		4	Digested		3
Dark colour		2	Liquified		0
Blackish		0			
(e) <u>Body Surface</u> (appearance)			(j) <u>Belly Wall</u>		
Bright shining		10	Normal fresh		10
Slight loss of brightness		8	Slight discoloration		8
Loss of brightness		6	More discoloration		6
Slight dull		5	Slightly digested		4
Dull and slight reddish		3	More digested		2
Moderately dull		2	Completely digested		0
Completely dull		0			

2.7.1.2 Chemical method: The quality of fish during spoilage was determined by estimation of Total Volatile Nitrogen (TVN) from TCA extract (TVN mgN/100 g sample by Conway microdiffusion technique (Conway and Byrne, 1933, modified by Paerson (1962))).

3. RESULTS AND DISCUSSION

3.1 Variation in Chemical Composition

3.1.1 Effect of species (Table 1a)

Composition of fish varied not only with species but also within the same species from one individual to another. This is mainly due to intrinsic characteristics of the species involved.

3.1.2 Effect of sex (Table 1b)

Moisture contents were higher, and protein and fat contents were lower in case of female fish with eggs. This is due to the fact that during egg formation fish expend a great deal of energy, the principal source of which is fat and protein. The two components are mobilized for gonad formation.

3.1.3 Effect of size (Table 1c)

Juvenile fish contained more protein but less fat than the adults in the same season. Such variation in composition is due to age and size. In the early stage the growth rate is rapid and a higher rate of protein anabolism takes place in younger fishes. Food taken by young fish is diverted to the building of new cells, i.e., mainly to protein.

3.1.4 Effect of anatomical portions (Table 1d)

The effect of anatomical portion was shown for only one species (*Glossogobius aureus*). Moisture, fat and ash content of the flesh are found to vary considerably in different parts. In the case of protein content, the variation was not pronounced. The ventral portion showed the highest fat content and lowest moisture content. Ash content was higher in the tail region due to a higher proportion of bones in this region.

3.1.5 Effect of season (Table 1e)

A fluctuation between summer and winter was observed in the individual biochemical components of the flesh. Variation in moisture and fat content with season was prominent. Higher fat contents and lower moistures were observed in winter than in summer. This variation may be attributed to physiological and environmental change, a.g., temperature and food availability.

3.2 Some Aspects of Quality Changes During Short-Term Preservation

3.2.1 Effect of using water weeds on spoilage changes

The effect of using water weeds was described in Table 2 (a-d) for summer.

It was found that four batches of fish kept under four different conditions had different rates of spoilage. It was observed that each batch of fish, kept in sunlight, spoiled earlier than corresponding batch of fish in shade. For all the batches, fish kept in shade with water weeds had the longer shelf life than others. This is perhaps due to the shading effect of moist water weeds which protected the fish from sunlight and high temperature.

Prawns without head kept slightly better than corresponding head-on prawns. This is due to the fact that bacterial load is always higher in the head region than in the body.

3.2.2 Effect of different storage temperature on spoilage changes (Table 3 (a-d))

Storage temperature has the profound effect on the rate of spoilage. All varieties of fish at superchilling temperature, -20°C , showed the longer shelf life than that 0°C , $+7^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $+15^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Thaputi fish stored at -20°C or 0°C (in ice) remained acceptable for about a week. It became unacceptable within 24 h of storage at $+15^{\circ}\text{C} \pm 2^{\circ}\text{C}$. At lower temperature and particularly below 0°C , the enzymatic and bacterial action was retarded to a great extent. The spoilage rate had a similar pattern among the different varieties. It is quite evident that there were great differences in spoilage rate and pattern for fish stored at four different temperatures, all other conditions for storage being essentially the same. It may be concluded that the differences in spoilage rate were solely due to the effect of storage temperature. The low TVN value, even in case of spoiled samples, was due to the leaching effect of melted ice water.

3.2.3 Effect of size on spoilage changes

Within the same species, the smaller fish spoiled earlier than the larger ones under the same storage conditions (room temperature, 29^o-30^oC). This is mainly due to the rapid penetration of spoilage bacteria in smaller fish with thinner skin and muscle.

3.2.4 Effect of species on spoilage changes

The rate of spoilage was different for different species within the same variety. Under the same storage conditions at room temperature (around 29^o-30^oC) in case of puti and bele fish, the order of spoilage rates of the species was as follows:

Puntius ticto > *Puntius stigma* > *Puntius sarana*/*Glossogobius giuris* > *Glossogobius melanota* > *Glossogobius sadanundia*.

This variation is probably due mainly to the difference in the intrinsic characteristics between the species and partly to the effect of size of the fish.

3.2.5 Effect of different containers on spoilage changes during ice storage (Table 4 (a-e))

It is evident from the results that bamboo baskets and wooden boxes, insulated with hoglamat, were found to be the most efficient in keeping fish in good condition and also maintaining a lower melting rate of ice. This is mainly due to the good insulating properties of hoglamat. Conversely, metallic containers showed the highest rate of spoilage. The higher thermal conductivity and higher rate of melting of ice contributed to the rapid spoilage of fish, all other conditions remaining the same.

4. MAJOR OBSERVATIONS

- (1) Composition of fish varies due to species, sex, size, anatomical position and season.
- (2) Spoilage can be retarded by keeping the fish in the shade and covered with water weeds.
- (3) Rate of spoilage is directly related to storage temperature. The higher the storage temperature the shorter the shelf life.
- (4) Rate of spoilage is higher in smaller fish than in larger ones.
- (5) Spoilage rate may be different for different species within the same variety.
- (6) Hoglamat-insulated bamboo baskets or wooden boxes were found to be most efficient in keeping iced fish in good condition.

5. REFERENCES

- AOAC (Association of Official Agricultural Chemists), Official method of Analysis. Washington, 1975 D.C., AOAC
- Fearson, D., The chemical analysis of food. Edinburgh, J.A. Churchill, 5th ed. 1962

Table 1a

Variation in proximate composition due to species

Name of fish	Moisture (g %)	Fat (g %)	Protein (g %)	Ash (g %)
<u>Puti</u>				
<i>Puntius sarana</i>	76.06	4.86	18.04	1.50
<i>Puntius stigma</i>	76.99	3.73	18.08	1.49
<i>Puntius tito</i>	77.62	2.22	19.10	1.61
<i>Puntius ohola</i>	77.12	4.14	17.44	1.53
<i>Puntius sophore</i>	77.92	3.36	17.76	1.51
<i>Puntius oonohomus</i>	77.17	3.99	17.51	1.58
<i>Puntius caucasicus</i>	77.69	2.86	18.52	1.48
<u>Bele</u>				
<i>Glossogobius giuris</i>	81.04	0.62	15.30	2.21
<i>Gobius melanostomus</i>	79.42	0.76	16.69	2.16
<i>Gobius personatus</i>	80.27	0.66	16.78	2.10
<i>Gobius sadanundio</i>	78.76	0.82	18.21	1.97
<i>Gobius crinitus</i>	80.59	0.66	16.12	1.89
<i>Eleotris butie</i>	79.94	0.71	16.38	2.09
<i>Eleotris fusca</i>	80.53	0.64	15.97	1.88

Table 1b

Variation in proximate composition due to sex

Name of the fish	Moisture (g %)		Fat (g %)		Protein (g %)		Ash (g %)	
	Female (egg)	Male	Female (egg)	Male	Female (egg)	Male	Female (egg)	Male
<i>Puntius stigma</i>	80.86	78.52	0.97	2.11	16.22	18.87	1.63	1.42
<i>Glossogobius giuris</i>	79.75	78.68	0.67	0.64	17.26	18.45	1.94	1.97
<i>Tilapia nilotica</i>	81.52	79.27	1.02	1.33	15.80	17.16	1.06	1.35
<i>Cyprinus carpio</i>	79.61	78.37	1.20	1.11	18.25	19.17	1.18	1.41
<i>Macrobrachium rosenbergii</i>	-	-	-	-	-	-	-	-
<i>Stromateus sinensis</i>	-	-	-	-	-	-	-	-

Table 1c

Variation in proximate composition due to size

Name of the fish	Moisture (g %)		Fat (g %)		Protein (g %)		Ash (g %)	
	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile
<i>Puntius stigma</i>	75.46	77.48	5.34	2.90	17.28	18.39	1.55	1.44
<i>Glossogobius giuris</i>	79.14	80.16	0.72	0.42	17.23	17.72	2.07	1.87
<i>Tilapia nilotica</i>	-	-	-	-	-	-	-	-
<i>Cyprinus carpio</i>	-	-	-	-	-	-	-	-
<i>Macrobraconium rosenbergii</i>	-	-	-	-	-	-	-	-
<i>Stromateus sinensis</i>	-	-	-	-	-	-	-	-

Table 1d

Variation in proximate composition
due to different anatomical portion of *Glossogobius giuris*

Anatomical portion	Moisture (g %)	Fat (g %)	Protein (g %)	Ash (g %)
Whole fish	80.28	0.71	16.23	2.20
Dorsal muscle	79.23	0.52	18.36	1.76
Ventral muscle	78.17	0.95	17.15	1.33
Tail muscle	81.08	0.67	15.75	2.03

Table 1e

Variation in proximate composition due to season

Name of the fish	Moisture (g %)		Fat (g %)		Protein (g %)		Ash (g %)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
<i>Puntius stigma</i>	75.46	78.52	5.34	2.11	17.28	18.87	1.55	1.42
<i>Glossogobius giuris</i>	80.50	80.06	0.61	0.81	16.09	16.36	2.21	2.18
<i>Tilapia nilotica</i>	81.01	79.18	1.04	1.31	16.31	16.65	1.14	1.27
<i>Cyprinus carpio</i>	80.78	77.20	1.30	1.00	17.54	19.88	1.05	1.53
<i>Macrobraconium rosenbergii</i>	78.30	78.15	1.00	1.25	19.41	19.42	1.30	1.20
<i>Stromateus sinensis</i>	70.10	69.00	9.00	10.02	19.50	19.60	1.42	1.40

Table 2

Effect of using water weeds on spoilage changes in sunlight and shade

Name of the sample	Storage period (h)						
	0	2	4	8	10	21	23
(a) Fish kept in sunlight with water weeds							
<i>Puntius stigma</i>	AC	HA	HA	A	JUA	UA	EUA
	TS	100	89	80	48	33	14
	TVN	17.84	19.21	23.33	35.69	48.04	210.0
<i>Glossogobius giuris</i>	AC	HA	A	MA	JA	JUA	- UA
	TS	100	78	55	41	24	- 5
	TVN	10.28	20.56	32.08	35.72	43.77	- 98.40
<i>Tilapia nilotica</i>	AC	HA	A	A	MA	-	EUA
	TS	100	85	70	58	-	28
	TVN	17.63	20.34	24.41	29.88	-	149.18
<i>Cyprinus carpio</i>	AC	HA	MA	MA	JUA	UA	UA
	TS	100	85	72	48	42	25
	TVN	16.27	17.63	21.70	35.26	54.25	198.01
<i>Macrobrachium rosenbergii</i> (head on)	AC	HA	HA	A	MA	JUA	EUA
	TS	100	92	88	60	42	12
	TVN	14.10	20.04	23.19	29.29	37.97	187.70
<i>Macrobrachium rosenbergii</i> (head off)	AC	HA	HA	A	MA	JUA	EUA
	TS	100	90	85	65	40	18
	TVN	14.10	18.95	21.10	26.04	35.80	177.94
(b) Fish kept in sunlight without water weeds							
<i>Puntius stigma</i>	AC	HA	A	JA	MUA	EUA	EUA
	TS	100	80	59	21	16	0
	TVN	17.84	20.59	26.08	43.92	57.64	214.11
<i>Glossogobius giuris</i>	AC	HA	A	MA	JA	MUA	- EUA
	TS	100	74	52	36	21	- 2
	TVN	10.28	22.90	34.08	37.25	44.57	- 106.24
<i>Tilapia nilotica</i>	AC	HA	A	MA	JUA	-	MUA
	TS	100	83	62	40	-	28
	TVN	17.63	24.41	28.48	48.82	-	214.28
<i>Cyprinus carpio</i>	AC	HA	A	JA	JUA	UA	-
	TS	100	74	58	36	26	-
	TVN	16.27	21.70	27.12	58.31	97.65	-
<i>Macrobrachium rosenbergii</i> (head on)	AC	HA	A	A	JA	JUA	EUA
	TS	100	90	80	65	40	18
	TVN	14.10	23.87	24.27	34.72	42.31	230.02
<i>Macrobrachium rosenbergii</i> (head off)	AC	HA	HA	A	JA	JUA	EUA
	TS	100	88	75	62	35	20
	TVN	14.10	24.95	22.10	32.55	39.06	183.87

AC = Acceptability
TS = Total score
TVN = TVN mgN/100 g

Table 2 (continued)

Name of the sample	Storage period (h)						
	0	2	4	8	10	21	23
(c) Fish kept in shade with water weeds							
<i>Puntius stigma</i>	AC	HA	HA	A	MA	JUA	EUA
	TS	100	96	88	65	47	2
	TVN	17.84	17.80	21.96	28.82	37.08	185.29
<i>Glossogobius giuris</i>	AC	HA	HA	A	A	MA	- JUA
	TS	100	88	72	56	37	- 12
	TVN	10.28	12.45	24.17	28.63	33.72	- 75.70
<i>Tilapia nilotica</i>	AC	HA	A	A	MA	- JUA	UA
	TS	100	95	90	70	- 48	39
	TVN	17.63	18.98	21.70	24.41	- 71.88	104.43
<i>Cyprinus carpio</i>	AC	HA	A	MA	MA	UA	- UA
	TS	100	91	85	65	47	- 31
	TVN	16.27	16.27	18.98	28.48	37.97	- 100.36
<i>Macrobrachium rosenbergii</i> (head on)	AC	HA	HA	A	A	JUA	EUA
	TS	100	89	80	68	58	15
	TVN	14.10	15.19	20.78	29.29	37.72	143.22
<i>Macrobrachium rosenbergii</i> (head off)	AC	HA	HA	A	A	A	EUA
	TS	100	85	70	60	52	20
	TVN	14.10	16.25	23.87	28.21	32.55	137.79 154.07
(d) Fish kept in shade without water weeds							
<i>Puntius stigma</i>	AC	HA	HA	A	JA	UA	EUA
	TS	100	92	83	52	37	06
	TVN	17.84	19.21	24.71	34.31	43.92	167.45
<i>Glossogobius giuris</i>	AC	HA	HA	A	MA	JA	- UA
	TS	100	81	61	44	32	- 9
	TVN	10.28	19.08	25.06	28.95	34.12	- 80.12
<i>Tilapia nilotica</i>	AC	HA	A	A	MA	- JUA	UA
	TS	100	88	77	70	- 39	31
	TVN	17.63	20.34	23.05	25.76	- 78.66	94.93
<i>Cyprinus carpio</i>	AC	HA	A	MA	JA	JA	- UA
	TS	100	85	73	50	46	- 34
	TVN	16.27	17.63	20.34	31.19	40.68	- 105.78
<i>Macrobrachium rosenbergii</i> (head on)	AC	HA	HA	A	JU	UA	EUA
	TS	100	85	78	70	55	15
	TVN	14.10	21.70	23.10	33.63	37.93	208.32 213.74
<i>Macrobrachium rosenbergii</i> (head off)	AC	HA	HA	A	A	A	EUA
	TS	100	92	82	70	60	15
	TVN	14.10	19.70	21.75	31.46	35.80	170.34

AC = Acceptability

TS = Total score

TVN = TVN mgN/100 g

Table 3

Effect of storage temperature on spoilage changes

Name of the sample	Storage period (days)									
	0	1	2	3	7	10	13	16	20	
<i>Pontius stigma</i>	(a) Storage temperature -2°C									
	AC	HA	A	A	MA	JA	JUA	MUA	EUA	EUA
	TS	100	84	72	57	42	36	31	22	15
	TVN	19.21	24.70	26.08	31.37	36.69	46.67	68.63	104.31	146.86
<i>Gloessogobius giaris</i>	AC	HA	A	A	MA	MA	JA	JUA	MUA	EUA
	TS	100	91	88	82	72	56	48	42	28
	TVN	13.28	16.39	16.09	24.67	28.82	30.41	47.40	60.07	80.64
<i>Tilapia nilotica</i>	AC	HA	-	A	MA	JA	JUA	UA	-	UA
	TS	100	-	87	74	57	48	40	-	32
	TVN	17.48	-	24.41	29.83	30.19	67.81	78.66	-	105.78
<i>Cyprinus carpio</i>	AC	HA	-	HA	A	MA	MA	JA	-	UA
	TS	100	-	90	80	77	68	64	-	45
	TVN	17.63	-	18.98	21.70	29.83	33.05	34.82	-	48.41
<i>Stromateus einensis</i>	AC	HA	-	-	HA	A	A	A	MA	JA
	TS	100	-	-	97	93	89	70	60	34
	TVN	9.76	-	-	16.27	20.61	24.95	31.46	35.80	61.84
<i>Pontius stigma</i>	(b) Storage temperature 0°C (ice storage)									
	AC	HA	A	MA	JA	UA	EUA			
	TS	100	83	73	51	35	18			
	TVN	17.84	19.21	21.96	24.71	21.96	37.08			
<i>Gloessogobius giaris</i>	AC	HA	A	MA	JUA	UA				
	TS	100	82	63	23	11				
	TVN	11.45	14.49	19.31	21.15	29.39				
<i>Tilapia nilotica</i>	AC	HA	MA	MA	JA	JA	UA			
	TS	100	84	76	64	56	44			
	TVN	16.27	17.63	18.98	18.80	19.08	23.05			
<i>Cyprinus carpio</i>	AC	-	-	-	-	-	-			
	TS	-	-	-	-	-	-			
	TVN	-	-	-	-	-	-			
<i>Stromateus einensis</i>	AC	HA	-	-	A	A	MA	UA	EUA	
	TS	100	-	-	85	78	65	40	12	
	TVN	9.76	-	-	16.27	18.44	27.12	78.12	164.92	

AC = Acceptability

TS = Total score

TVN = TVN mgN/100 g

Table 3 (continued)

Name of the sample	Storage period (days)								
	0	1	2	3	7	10	13	16	20
<i>Puntius stigma</i>	(c) Storage temperature +7°C ± 1°C								
	AC	HA	A	MA	UA	MUA	EUA		
	TS	100	72	59	45	31	06		
	TVN	19.21	26.08	33.89	48.02	137.25	263.62		
<i>Glossogobius giuris</i>	AC	HA	A	JA	MA	UA	MUA	MUA	EUA
	TS	100	86	82	74	64	52	38	30
	TVN	13.28	16.43	20.73	28.49	42.27	61.21	84.79	102.46
<i>Tilapia nilotica</i>	AC	HA	-	MA	JUA	UA	UA	MUE	-
	TS	100	-	84	47	29	24	16	-
	TVN	17.98	-	27.12	48.82	80.01	116.63	157.32	-
<i>Cyprinus carpio</i>	AC	HA	-	MA	MA	JUA	UA	MUA	-
	TS	100	-	86	68	49	38	28	-
	TVN	17.63	-	21.70	29.83	36.61	94.93	174.95	-
<i>Stromateus sinensis</i>	AC	HA	-	-	A	MA	UA	MUA	EUA
	TS	100	-	-	85	50	34	24	18
	TVN	9.76	-	-	31.46	50.99	72.69	151.05	257.14
<i>Puntius stigma</i>	(d) Storage temperature +15°C ± 2°C								
	AC	HA	UA	EUA	EUA				
	TS	100	40	10	02				
	TVN	19.21	85.10	211.37	-				
<i>Glossogobius giuris</i>	AC	HA	MA	UA	EUA				
	TS	100	63	42	30				
	TVN	13.28	39.87	80.83	107.98				
<i>Tilapia nilotica</i>	AC	HA	-	UA	EUA	EUA			
	TS	100	-	38	10	2			
	TVN	17.98	-	43.40	221.06	-			
<i>Cyprinus carpio</i>	AC	HA	-	UA	MUA	EUA	EUA		
	TS	100	-	34	16	3	0		
	TVN	17.63	-	116.63	347.2	401.45			
<i>Stromateus sinensis</i>	AC	HA	-	-	UA	EUA	EUA		
	TS	100	-	-	40	22	12		
	TVN	9.76	-	-	87.97	137.79	365.64		

AC = Acceptability

TS = Total score

TVN = TVN mgN/100 g

Table 4

Effect of containers on spoilage changes during ice storage

Different containers used	Storage period (days)						
	0	1	3	6	10	14	15
(a) <i>Puntius stigmus</i>							
Fish in polyethylene bag kept in wooden box with ice	AC	HA	A	MA	JA	UA	MUA
	TS	100	84	69	56	41	26
	TVN	16.47	19.21	23.05	28.52	46.66	96.08
Fish without polyethylene bag kept in wooden box with ice	AC	HA	A	MA	JA	UA	EUA
	TS	100	83	73	51	35	18
	TVN	17.84	19.21	21.96	24.71	21.96	37.08
Fish in polyethylene bag kept in bamboo basket with ice	AC	HA	A	A	JUA	UA	EUA
	TS	100	81	71	48	32	3
	TVN	17.84	21.96	23.33	24.71	21.96	31.58
Fish without polyethylene bag kept in bamboo basket with ice	AC	HA	A	A	JUA	UA	EUA
	TS	100	81	71	48	32	3
	TVN	17.84	21.96	23.33	24.71	21.96	31.58
Fish in polyethylene bag kept in metallic container with ice	AC	HA	A	MA	UA	MUA	EUA
	TS	100	76	59	44	28	4
	TVN	16.47	26.08	30.20	35.69	61.76	129.2
Fish without polyethylene bag kept in metallic container with ice	AC	HA	A	A	UA	MUA	EUA
	TS	100	78	65	45	22	2
	TVN	17.84	18.95	20.59	15.09	10.96	6.86
(b) <i>Glossogobius aureus</i>							
Fish wrapped in hoglamet kept in wooden box with ice	AC	HA	A	MA	JA	MUA	UA
	TS	100	86	71	32	25	18
	TVN	11.45	12.07	13.28	26.56	32.07	
Fish without wrapping in hoglamet kept in wooden box with ice	AC	HA	A	MA	JUA	UA	
	TS	100	82	63	23	11	
	TVN	11.45	14.49	19.31	21.15	29.39	
Fish wrapped in hoglamet kept in bamboo basket with ice	AC	HA	A	MA	JA	UA	
	TS	100	80	65	26	14	
	TVN	11.45	12.56	16.90	28.59	33.64	
Fish without wrapping in hoglamet kept in bamboo basket with ice	AC	HA	A	MA	JUA	EUA	
	TS	100	76	58	20	6	
	TVN	11.45	13.59	22.94	28.98	31.22	

AC = Acceptability
TS = Total score
TVN = TVN mgN/100 g

Table 4 (continued)

Different containers used	Storage period (days)						
	0	1	3	6	10	14	15
(c) <i>Tilapia nilotica</i>							
Fish in polyethylene bag kept in wooden box with ice	AC	HA	A	MA	JA	JUA	UA
	TS	100	84	72	57	45	38
	TVN	16.27	21.70	24.76	27.12	29.80	34.25
Fish wrapped in boglamat kept in wooden box with ice	AC	HA	MA	MA	JA	JA	UA
	TS	100	84	76	64	56	44
	TVN	16.27	17.63	18.98	18.80	19.07	23.05
Fish in polyethylene bag kept in bamboo basket with ice	AC	HA	A	MA	JA	JA	UA
	TS	100	87	69	53	50	46
	TVN	16.27	24.41	25.76	29.33	30.26	34.25
Fish wrapped in boglamat kept in bamboo basket with ice	AC	HA	A	A	MA	JUA	UA
	TS	100	83	72	55	46	42
	TVN	16.27	16.49	18.98	24.44	20.34	29.83
Fish in metal container with ice	AC	HA	A	MA	JA	JUA	UA
	TS	100	88	74	58	45	39
	TVN	16.27	17.63	17.63	13.65	10.85	10.41
(d) <i>Macrobrachium rosenbergii</i> (head on)							
Fish in polyethylene bag kept in wooden box with ice	AC	HA	A	A	MA	JA	MUA
	TS	100	85	72	60	43	30
	TVN	11.93	23.87	15.19	29.29	59.67	92.22
Fish without polyethylene bag kept in wooden box with ice	AC	HA	HA	A	MA	JA	UA
	TS	100	88	75	62	55	40
	TVN	11.39	17.36	21.70	27.12	34.72	48.82
Fish in polyethylene bag kept in bamboo basket with ice	AC	HA	A	A	A	JA	UA
	TS	100	88	78	68	55	28
	TVN	11.93	23.87	17.36	34.72	85.71	166.09
Fish without polyethylene bag kept in bamboo basket with ice	AC	HA	A	A	MA	JA	UA
	TS	100	88	72	64	50	30
	TVN	11.93	19.53	14.10	22.78	26.04	43.40

AC = Acceptability

TS = Total score

TVN = TVN mgN/100 g

Table 4 (continued)

Different containers used	Storage period (days)						
	0	1	3	6	10	14	15
Fish in polyethylene bag in wooden box with ice	(a) <i>Macrobrachium rosenbergii</i> (head off)						
	AC	HA	HA	A	A	JA	UA
	TS	100	92	85	70	55	30
	TVN	11.93	20.61	14.10	27.12	50.99	74.86
Fish without polyethylene bag kept in wooden box with ice	AC	HA	HA	A	A	MA	JA
	TS	100	90	75	63	52	38
	TVN	11.93	16.27	19.53	26.04	33.63	44.57
Fish in polyethylene bag kept in bamboo basket with ice	AC	HA	HA	A	JA	JUA	UA
	TS	100	90	78	60	50	30
	TVN	11.93	20.70	16.27	28.21	83.54	111.75
Fish without polyethylene bag kept in bamboo basket with ice	AC	HA	HA	A	MA	JA	MUA
	TS	100	88	73	60	42	25
	TVN	11.93	11.36	13.02	20.61	23.87	40.17

AC = Acceptability

TS = Total score

TVN = TVN mgN/100 g

MESOPHILIC SPOILAGE OF BAY TROUT (*Arripis trutta*), BREEM (*Acanthopagrus butcheri*)
AND MULLET (*Aldrichetta forsteri*)

by

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ABSTRACT

A handling procedure typical of those in tropical countries was simulated in this storage trial, i.e., "partial icing" to around 10°C during fishing, then marketing at ambient temperature (29°C). Monitoring sensory changes during storage resulted in rejection at 13 h at 29°C following 4 days at 10°C; at which time odour panel identified ammoniacal faecal odours on cooked fish: bay trout (*Arripis trutta*), bream (*Acanthopagrus butcheri*) and mullet (*Aldrichetta forsteri*). Total bacterial, VRBCA and H₂S producing counts also monitored were, at rejection 10⁸-10⁹/g, 10⁷-10⁸/g, and 10⁷-10⁸/g respectively. VRBCA and H₂S producers counts represented 15-42% and <1-7% total count, respectively. After 4 days at 10°C VRBCA isolates (29°C incubation) comprising predominantly *Pseudomonadaceae* (48%), *Vibrionaceae* (32%), *Enterobacteriaceae* (10%) and *Achromobacter-Alcaligenes* group (10%) changed at rejection to predominantly *Vibrionaceae* (60%), *Enterobacteriaceae* (35%) and *Pseudomonadaceae* (5%). Principal species was *Aeromonas hydrophila* (50%) at rejection. All *Aeromonas hydrophila* (50%) at rejection. All *Aeromonas hydrophila* isolated spoiled inoculated sterile muscle blocks at 29°C producing ammoniacal faecal odours similar to those on naturally spoiled fish at 29°C, in addition were TMAO producers and volatile sulphides producers at 29°C; and hence was the spoiler of "partially chilled" fish.

1. INTRODUCTION

Even though immense wastage and losses were reported in various tropical countries (Disney, 1976; Kamari, Idrus and Merican, 1977; Kamari and Merican, 1978; Embuscado and Mateo, these proceedings), little is known and researched on the spoilage mechanisms associated with typical tropical handling procedure and therefore to account for these losses and wastages. Moreover, fish landed from the tropics comprised 60% of the world's catch (FAO, 1981) emphasizing further the necessity to comprehend the spoilage mechanisms of fish handled in the tropics.

The present study simulated a common practice, i.e., icing of catch in the tropics, where the use of crushed block ice is used for storing catches during fishing. However, such icing practices on board fishing vessels are usually inadequate particularly when ice is expensive and scarce as in rural communities.

Moreover, vessel capacity limits the amount of ice that can be carried on board, particularly as fishing vessels are usually "small boat" types, mostly under 5 t inboard powered boats, outboard 20 to 40 hp boats (Kamari, Idrus and Merican, 1977) or sailboats.

Sorting the catch usually precedes storing. Sorting after capture is often on open decks at ambient temperature (25-40°C) and this involved exposing the fish to the sun for considerable periods up to 4 h (Kamari, Idrus and Merican, 1977). After sorting, the catch is stored in ice in wooden holds on board the "bigger" boats or on open deck of "smaller" boats. The wooden holds used are usually unigulated; therefore, ice melts away faster, further enhanced by the high temperatures at sea (30°C) in the tropics and the effect of sun exposure.

Storage time in holds, governed by vessel capacity, vary from 8-10 h in Malaysia (Kamari and Merican, 1978) to 4-5 days in Indonesia or to 7 days in Sri Lanka (Gunasekare and Lentz, 1965) up to periods of 5-10 days in Pakistan (Zuberi and Quadri, 1980). Because of the fishing period, inadequate icing and accelerated ice melting rate due to non-insulation of holds, storage on open decks and exposure directly to sun, the catch when landed at the harbour would be at approx. 10-12°C with ice barely visible. Re-icing, moreover, is usually not carried out on further handling in the distribution system (Kamari and Merican, 1978; Zuberi and Quadri, 1980; Embuscado and Mateo, these proceedings) and eventual marketing is carried out at ambient (25-40°C) temperature.

The very few studies on spoilage at ambient tropical temperatures (25-40°C) before 1984 unfortunately, did not relate degradative pathways of iced temperate fish and to tropical fish such as TMAO reduction to TMA for fishy spoilage odours (Laycock and Ragier, 1971; Janssen and Schultz, 1980), and volatile sulphides production such as H_2S , methyl mercaptan and dimethyl sulphide from S-amino acids, cysteine and methionine (Harbert, Ellis and Shawan, 1975; Herbert and Shawan, 1975).

As a result, this research project studied the spoilage of tropical fish resulting from handling fish at ambient (29°C) after "partial chilling" (10°C for 4 days) at sea. Bacterial and microfloral changes were studied quantitatively and qualitatively during storage. Biochemical activities, e.g., TMAO reduction, volatile sulphides production and "off-odours" on starila muscles were criteria used to identify the mesophilic spoiler.

2. MATERIALS AND METHODS

2.1 Fish Raw Materials

Bay trout (*Arripis trutta*), bream (*Acanthopagrus butcheri*) and mullet (*Aldrichetta forsteri*) were caught by beach seining off Hobson's Bay, Victoria; where the water and air temperatures at time of catch were 18 and 15°C, respectively. After transporting within 1/2 h fish were sacrificed upon arrival at the laboratory.

2.2 Storage

Each species (5-6 fish) was placed on a raised plate which was surrounded by (but not in contact with) moistened towellings to maintain humidity in a tray, covered with aluminium foil, and kept in a cabinet at 10°C for 4 days, then transferred to an incubator held at 29°C.

2.3 Sampling

Sensory and microbiological analyses were carried out on at least 3 fish per species at 4-6 intervals for fish incubated at 29°C.

2.4 Sensory Analysis

Raw fish was scored for quality on a demerit point scoring system devised by Dr Olley (CSIRO, personal communication). Scoring fresh fish gave a minimum score of zero, whereas spoiled fish scored a maximum 39 when whole, and 25 when decapitated due to 7 scores exclusions each from eyes and gills. Scores were expressed as a percentage of total raw score for comparison between sensory changes in whole and decapitated fish.

Excised tail flesh cooked in aluminium foil at 250°C for 15 min was judged for odour, flavour, texture and general acceptability on a 5-point scoring system. The limit of acceptability was when averaged scores of all judgements added ≤ 2 .

2.5 Microbiological Analysis

Samples excised from stomach flaps (13-18 g) placed in stomach bags to which were added sterile peptone water (0.1%) for a decimal dilution were homogenized in a Colworth "stomacher" for 60 s. Serial dilutions were made, 0.1 ml inoculum was pipetted on the surface of each half of media plate. Analyses in duplicate for each dilution were made on: nutrient agar (Oxoid CM 3) for total bacterial count; modified peptone-iron agar (Sumner and Gorczyca, 1981; modified from Levin, 1968) for hydrogen-sulphide (H_2S) producers (black colonies) and VRBGA (Oxoid CM 485) for *Enterobacteriaceae* count. Plates were then incubated at 29°C for 3 days when colonies counted from each half plate were added, averaged and expressed as count/g.

2.6 Identification of Isolates

A total of 40 single colonies per species were isolated from initial and rejection VRBGA plates and classified according to the scheme of Lea, Hendria and Shawan, 1979. Isolates were incubated at 29°C.

A total of 10 tests were used: gram stain (Lillie's modification), oxidase test (Kovacs, 1956), oxidative and fermentative (O.F.) test (Hugh and Leifson, 1953), motility tests, sensitivity to the vibriostatic agent O/129, growth at 37°C, aesculin hydrolysis (Cowan, 1974), arginine, lysine and ornithine decarboxylation.

2.7 Biochemical Tests for Determining Spoilage Bacteria

Isolates from rejection plates were subjected to two tests at 29°C, i.e., TMAO reduction (Huss, personal communication) and volatile sulphides production from methionine medium (0.1 g/100 ml broth) and cysteine medium (0.02 g/100 ml broth, McManis, Gibbs and Patterson, 1978).

2.8 "Off" Odours on Inoculated Sterile Muscles

Sterile muscle blocks were obtained by the method of Herbert (1970). After inoculation, the sterile muscle blocks were subjected to similar incubating conditions as was the fish, viz 10°C for 4 days followed by incubation at 29°C. Isolates tested were *Aeromonas hydrophila* (30) and *Enterobacteriaceae* (16). Uninoculated sterile muscle blocks were used as controls. Assessments were carried out after 20 h at 29°C and again within the next 24 h for isolates that did not produce any odours within the first 24 h. An odour panel of 12 judges was presented with 3 coded inoculated sterile muscle blocks. The tests were arranged such that either two *Aeromonas hydrophila* inoculated muscles were compared against an *Enterobacteriaceae* inoculated muscle or an uninoculated muscle in a duo-trio test.

3. RESULTS

Spoilage of 3 marine species studied: bay trout, bream and mullet as indicated by the first detectable ammoniacal faecal odours along with "mushy" texture giving an average cooked score of <2, occurred after 13 h at 29°C following "partial chilling" (10°C for 4 days, Table 1). Visually and organoleptically, no marked decrease in quality of "partial chilled" fish was apparent after 4 days at 10°C (Table 1) at which time fish accumulated a sensory raw score of 22-36% after a rise from zero for fresh fish (Table 2). Subsequently changing incubation temperature to 29°C, however, caused a jump in sensory raw score to 50% after only 5 h, accompanying a loss in fish sheen, rapid gills deteriorations and progressive sinking of ayes. Eventually after 13 h at 29°C the rejected spoiled fish had a sensory raw score of 78-81%. Percentages of raw scores being that of the maximum total raw score of 39 for whole fish and 25 for decapitated fish (7 scores exclusion each from ayes and gills) for comparison between the three fish species examined.

3.1 Microbiological Analysis

Total counts at initial levels of 10^3 , 10^4 and 10^5 /g of bay trout, bream and mullet, respectively increased 2-3 log scales to 10^6 - 10^7 /g after 4 days at 10°C. Subsequently, total counts further increased 1-2 log scale to 10^8 - 10^9 /g at rejection at 29°C for all three fish species (Table 3).

H_2S producers initially at <10/g increased 2-5 log scales to 10^3 , 10^4 and 10^6 /g after 4 days at 10°C constituting 2, <1 and 2% total count of bay trout, bream and mullet, respectively. H_2S producers counts further rose 2 log scales to 10^5 /g, 10^6 /g and 10^8 /g at rejection at 29°C constituting 22, <1% and 7% total count of the three fish in the order mentioned previously.

VRBGA counts initially at <10- 10^2 /g rose 4-5 log scales to 10^5 - 10^6 /g after 4 days at 10°C. This range represented 27, 24 and 5% of total count of bream, bay trout and mullet respectively. VRBGA counts further rose to 10^7 - 10^8 /g at rejection at 29°C representing 24, 47 and 15% total count of bay trout, bream and mullet respectively (Table 3). Graphing (Figure 1) bacterial counts showed VRBGA counts at higher levels than H_2S producers throughout storage on all three fish examined. These higher counts enumerated on VRBGA than on peptone iron agar accordingly warranted the isolation of colonies from VRBGA plates for identification.

3.2 Isolation and Identification of Isolates

All 120 isolates from VRBGA plates incubated at 29°C of the three fish species tested were gram negative rods. All were classified into four groups according to the schemes of Lee, Hendrie and Shewan, 1979 and Lupage, et al., 1979.

Initial microflora comprising predominantly of *Pseudomonadaceae* (55%), *Achromobacter-Alcaligenes* group (27%) and *Vibrionaceae* (18%) changed 4 days at 10°C to *Pseudomonadaceae* (48%), *Vibrionaceae* (32%), *Enterobacteriaceae* (10%) and *Achromobacter-Alcaligenes* group (10%). However, at rejection, *Vibrionaceae* dominated at 60%, with *Enterobacteriaceae* at 35% and *Pseudomonadaceae* at 5% (Table 6). Similar microflora distribution initially, after 4 days at 10°C and at rejection was isolated from all three marine fish species examined (Table 6). Further investigation on *Vibrionaceae* isolates revealed the dominant genus as *Aeromonas* at 10°C for 4 days and at rejection (Table 5). Of these *Aeromonas*, the dominant species was *Aeromonas salmonicida* (79%) initially, i.e., after 4 days at 10°C. However, at rejection about 90% of the isolates examined were identified as *Aeromonas hydrophila*.

3.3 Biochemical Activity

The results in Table 6 showed all *Aeromonas hydrophila* isolates were biochemically active; all reducing TMAO to TMA at 29°C and producing volatile sulphides, viz. methyl mercaptan and dimethyl sulphides at 29°C. In addition, only 17% were also H_2S producers. In comparison, although *Enterobacteriaceae* isolates similarly were TMAO reducers (90%) and volatile sulphides producers (57%) at 29°C, all were also H_2S producers.

3.4 Inoculation of Sterile Muscle Blocks

Starila muscle blocks inoculated with *Aeromonas hydrophila* spoiled within 24 h at 29°C during which time odour panelists identified similar ammoniacal, putrid, pungent and faecal like "off-odours" on which naturally spoiled fish were rejected (Table 7). In contrast, no "off-odours" were evident on sterile muscle blocks inoculated with *Enterobacteriaceae* isolates within the same period. However, although edge (38%) were later detected as spoiled with "off-odours" produced within the following 24 h at 29°C, only "pungent" odours produced were identical to those on the naturally spoiled fish.

4. DISCUSSION

Shelf lives (approx. 13 h) obtained similarly by all three fish species studied showed that shelf life was not affected by fish species difference. Comparing with another mesophilic study (Estrada, et al., these proceedings) showed that the three species studied had approx. 5-6 h longer shelf life at ambient temperature (29°C) than tropical whiting, *Sillago maculata* also at 29°C although rejection criteria: putrid ammoniacal cooked odour, "mushy" texture and repulsing bitter cooked flegur, were similar. A possible explanation is that the species studied were from temperate waters (18°C) whereas *Sillago maculata* was from tropical waters and, therefore, have differing microflora. Microflora differences suggested by numerous workers (Diney, Cols and Jones, 1974; Nair and Dani, 1975; Diney, 1976; Shewan, 1977) where that natural mesophilic flora on tropical fish are better adapted for growth at 25-40°C than the more psychrophilic flora on temperate fish probably explained the shorter shelf life of tropical fish than temperate fish at 25-40°C. Thus, applying such studies on temperate fish to tropical fish may warrant a few hours deduction in shelf life.

The initial total counts (10^3 - 10^5 /g) and the rise to 10^8 - 10^9 /g were similar to those established for other temperate marine fish (Gillepie and MacRae, 1975; Shewan, 1977) and also for both tropical marine and freshwater fish (Estrada, et al., Putro, et al., and Saleh, et al., these proceedings).

The proportion of H_2S producers to total count at rejection (<1-7%) obtained were lower than those (10-30%) quoted on spoiled iced temperate fish (Chai, et al., 1968; Herbert, Ellis and Shewan, 1971). Other studies (Estrada, et al., and Saleh, et al., these proceedings), like the present study, also found similar low numbers of H_2S producers, contrary to the usual domination at iced spoilage of temperate and tropical fish (Shewan, 1977; Sumner and Gorczyca, 1981). Therefore, the role of H_2S producers was doubted in mesophilic spoilage.

The findings of VRBGA counts exceeded H_2S producers counts and had a greater incremental growth rate than total counts, therefore, circumstantiated the importance of VRBGA colonies as spoilers.

Aeromonas hydrophila isolated was capable of all three reactions: TMAO reduction, volatile sulphides production and similar off-odour production on muscle blocks to naturally spoiled fish; confirmation of a spoiler. Its presence, at spoilage, were similarly reported for tropical fish at 0°C seawater (42%), at 10°C CSW storage gave 25% (Barile, et al., these proceedings) and on spoiled tropical fish subjected to 9 to 12-h delay, before icing (Barile, et al., these proceedings). The ammoniacal off-odour produced by *Aeromonas hydrophila* were also reported to be produced by *Vibrionaceae* isolated from commercially handled fish on trypsin-treated fish homogenate serum at 20°C (Gillaspie and MacRae, 1975).

The most significant feature in the isolation history of *Aeromonas hydrophila* is none so far of its role as a fish spoiler, until recently and in this study. The significant features in this study were occurrences of *Aeromonas hydrophila* in coastal marine waters in contrast to the usual isolation from freshwater environments (Vezina and Desrochers, 1971; Shotts, et al., 1972; Thorpe and Roberts, 1972; Hazan, et al., 1978) and the implications, for the first instance, as a fish spoiler of marine species subjected to handling conditions typical of those in tropical countries (e.g., "partial chilling"). These implications, along with infrequent H_2S production by this organism plus the finding of low numbers of H_2S producers, high VRBGA counts and the very low numbers (5%) of *Pseudomonadaceae* isolated, specifically the absence of *Alteromonas putrefaciens* at rejection, suggested the possible irrelevance of using iron agar (Levin, 1968) particularly for enumerating black colonies (H_2S producer) in mesophilic spoilage.

Moreover, the isolation of *Enterobacteriaceae* as the second dominant (35%) microflora at rejection and the capabilities of these isolates for all three mentioned bio-activities inferred the ability of Gram-negative mesophiles, of which a large portion of *Enterobacteriaceae* are, to have a role in mesophilic spoilage.

Interestingly, *Enterobacteriaceae* did not constitute any of the initial microflora isolated in the present study or that of Shewan (1977) and Barile, et al., (these proceedings) on fish from warm waters (20-30°C). Thus, the greater importance of VRBGA, especially for *Enterobacteriaceae* counts, is apparent. Lastly, the isolation and implications of *Aeromonas hydrophila* as a spoiler suggested

the possible greater usage and importance of media for the presumptive enumeration of this organism developed by Shotts and Rinler (1973) or by Kaper, *et al.* (1979).

5. REFERENCES

- Chai, T., *et al.*, Detection and incidence of specific species of spoilage bacteria on fish.
1968 2. Relative incidence of *Pseudomonas putrefaciens* and fluorescent *Pseudomonas* on
hadcock fillete. Appl. Microbiol., 16:1738-41
- Cowan, S.T., Cowan and Steel's manual for the identification of medical bacteria. Cambridge,
1974 Cambridge University Press, 2nd ed.
- Disney, J.G., Spoilage of fish in the tropics. In Proceedings of the First annual tropical and
1976 subtropical fisheries technological Conference. College Station, Texas A&M University,
pp. 23-39
- Disney, J.G., R.C. Cole and N.R. Jones, Considerations in the use of tropical fish species. In
1974 Fishery products, edited by R. Kreuzer. Surrey, England, Fishing News (Books) Ltd.,
for FAO pp. 329-37
- FAO, Yearbook of fishery statistics. Annuaire statistique des pêches. Anuario estadístico de
1981 pesca, 1980. Catchee and landings, Capturee et quantités débarquées. Capturæ y
desambarques. Yearb. Fish. Stat./Annu. Stat. Pêches/Anu. Estad. Pesca, (50):39
- Gillepie, N.C. and I.C. MacRae, The bacterial flora of some Queensland fish and its ability to
1975 cause spoilage. J. Appl. Bacteriol., 39:91-100
- Gunasekara, L.D. and A.W. Lantz, Investigations into the keeping qualities of ungutted fish from
1965 trawlers. Prog. Rep. Biol. Technol. Fish. Res. Sri Lanka, (1):4 p.
- Hazen, T.C. *et al.*, Prevalence and distribution of *Aeromonas hydrophila* in the US. Appl. Environ.
1978 Microbiol., 36:731-8
- Herbert, R.A., A study of the roles played by bacterial and autolytic enzymes in the production of
1970 volatile sulphur compounds in spoiling North Sea cod (*Gadus morhua*). Ph. D. Thesis,
University of Aberdeen
- Herbert, R.A. and J.M. Shewan, Precursors of the volatile sulphides in spoiling North Sea cod
1975 (*Gadus morhua*). J. Sci. Food Agric., 26:1195-202
- Herbert, R.A., J.R. Ellis and J.M. Shewan, Isolation and identification of a volatile sulphides
1975 produced during chill storage of North Sea cod (*Gadus morhua*). J. Sci. Food Agric.,
26:1187-94
- Herbert, R.A., *et al.*, Bacteria active in the spoilage in certain seafoods. J. Appl. Bacteriol.,
1971 34:41-50
- Hugh, R. and E. Lefson, The taxonomic significance of fermentative versus oxidative metabolism of
1953 carbohydrates by various Gram-negative bacteria. J. Bacteriol., 66:24-6
- Jeneen, M.N. and E. Schultz, Utilization of iron agar in determining the freshness of wet fish.
1980 Dan. Vet. Tidsskr., 68(8):314-81
- Jones, N.R., *et al.*, Rapid estimation of hypoxanthine concentrations as indices of the freshness of
1964 chill-stored fish. J. Sci. Food Agric., 15:664
- Kamari, A. and Z. Merican, Iced storage of Malaysian fish. Proc. IPFC, 18(3):197-212
1978
- Kamari, A., A.Z. Idrus and Z. Merican, The handling and transportation of fish in Malaysia. In
1977 Proceedings of the Conference on the handling, processing and marketing of tropical fish.
London, Tropical Products Institute, pp. 115-9
- Kaper, J., *et al.*, Medium for the presumptive identification of *Aeromonas hydrophila* and
1979 *Enterobacteriaceae*. Appl. Environ. Microbiol., 38(5):1023-6
- Kovace, N., Identification of *Pseudomonas pyocyanus* by oxidase reaction. Nature, Lond., 178:703
1956

- Lapaga, S.P., et al., Biochemical identification of *Enterobacteriaceae*. In Identification methods for microbiologists, edited by F.A. Skinner and D.W. Lovelock. London, Academic Press, 2nd ed. 1979
- Laycock, R.A. and L.W. Regier, TMA producing bacteria on haddock filllets during refrigerated storage. J.Fish.Res.Board Can., 28(3):305-9 1971
- Lee, J.V., M.S. Hendrie and J.S. Shewan, Identification of *Aeromonas*, *Vibrio* and related organisms. In Identification methods for microbiologist, edited by F.A. Skinner and D.W. London, Academic Press, pp. 151-66, 2nd ed. 1979
- Larka, P., R. Adams and L. Ferber, Bacteriology of spoilage of fish muscle. 3. Characterization of 1965 spoilage. Appl.Microbiol., 13:625-30
- Levin, R.E., Detection and incidence of specific species of spoilage bacteria on fish. 1. Method- 1968 ology. Appl.Microbiol., 16:1734-7
- McMeekio, T.A., P.A. Gibbs and J.T. Petterson, Detection of volatile sulphide producing bacteria isolated from poultry-processing plants. Appl.Environ.Microbiol., 35(6):1216-8 1978
- Nair, R.B. and N.P. Dani, Technology of utilisation of freshwater fish. In Proceedings of the 1975 Symposium on fish processing industry in India. Mangalore, India, Sharda Press, pp.20-2
- Shewan, J.M., The bacteriology of fresh and spoiling fish and the biochemical changes induced by 1977 bacterial action. In Proceedings of the Conference on the handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 51-6
- Shimazono, H., Distribution of 5' - ribonucleotides in foods and their application to foods. Food 1964 Technol., 18(3):36, 41-5
- Shotte, E.B., Jr. and R. Rimler, Medium for the isolation of *Aeromonas hydrophila*. Appl.Microbiol., 1973 26(4):550-3
- Shotts, E.M., et al., *Aeromonas* induced deaths among fish and reptiles in an eutrophic inland lake. 1972 J.Am.Vet.Med.Assoc., 161:603-7
- Summar, J.L. and E. Gorczyca, Effect of vacuum-packaging on the shelf-life of fish held either in 1981 ice or at 4-6°C. Sci.Tech.Food/Refrig.Sci.Technol., Paris, (1981-4):365-70
- Thorp, J.E. and R.J. Roberts, An *aeromonad* epidemic in the brown trout. J.Fish Biol., 4:441-51 1972
- Vezine, R. and R. Destrochers, Influence d'*Aeromonas hydrophila* chez le perche, *Perca fluviatilis* 1971 Mitchell. Can.J.Microbiol., 17:1101-3
- Zubari, R. and R.B. Quedri, Bacteriological status of fish and shrimps at landing on fish harbour 1980 and local retail markets in Karachi, Pakistan. Pak.J.Sci.Ind.Agr., 23(5):196-200

Table 1

Organoleptic changes in "partially chilled" mullet (*Aldrichetta forsteri*)

Time	Changes in cooked flesh			Average Cooked Score
	Odour	Flavour	Texture	
<u>Days at 10°C</u>				
0	"seaweedy"	sweet "seaweedy"	firm, moist	5
4	"fresh"	alightly bland	firm, elightly dry	4
<u>Hours at 29°C</u>				
5	weak "fishy"	bland	fairly firm	3 1/2
10	"fishy"	bland to stale	softening	2 1/2
13 1/4	pungent ammoniacal "faecal-like"	repulsive, putrid	"mushy"	1

Similar organoleptic changes were obtained on partially chilled bay trout (*Arripis trutta*) and bream (*Acanthopagrus butcheri*)

Table 2

Raw scores of "partially chilled" marine fish

Hours at 29°C	Raw scores (% maximum total raw score*)					
	Mullet (<i>Aldrichetta forsteri</i>)		Bream (<i>Acanthopagrus butcheri</i>)		Bay trout (<i>Arripis trutta</i>)	
0	9	(36)	10	(26)	8	(22)
5	14	(56)	17	(44)	17	(44)
10	16	(64)	24	(62)	22 1/2	(58)
13	19 1/2	(78)	30 1/2	(78)	31 1/2	(81)
* Maximum total raw score						
	25		39		39	

Table 3

Shelf lives and bacterial counts (25°C incubation) for "partially iced" marine fish

Marine fish	Shelf life at 25°C (Hours)	Bacterial counts (No./g (% of total count))								
		Total counts			VRBCA counts			H ₂ S producers		
		I*	M*	R*	I*	M*	R*	I*	M*	R*
Bay trout (Glenoid trutta)	13 1/2	2.8x10 ³	3.0x10 ⁶	1.7x10 ⁸	<10 (<1)	7.2x10 ⁵ (24)	4.0x10 ⁷ (24)	10 (<1)	4.8x10 ⁴ (2)	2.3x10 ⁶ (2)
Bream (Abramis brama) (Dutchman)	13 1/4	1.9x10 ⁴	3.6x10 ⁶	2.0x10 ⁸	<10 (<1)	9.6x10 ⁵ (27)	9.3x10 ⁷ (42)	10 (<1)	5.0x10 ³ (<1)	5.2x10 ⁵ (<1)
Mullet (Mullus barbatus) (Mullet)	13	2.0x10 ⁵	5.8x10 ⁷	2.0x10 ⁹	4.9x10 ² (<1)	2.9x10 ⁶ (15)	3.0x10 ⁸ (15)	10 (<1)	1.0x10 ⁶ (2)	1.4x10 ⁸ (7)

*I = initial)

*M = 10°C/4 days)

*R = rejection)

Table 4

Microflora isolated from VRBCA from mullet, bream and bay trout ^{B/}

PERIOD	NUMBER	Microflora No. (%)			
		<i>Pseudomonas</i>	<i>Vibrio</i>	<i>Enterobacteriaceae</i>	Others
Initial ^{B/}	60	29 (48)	19 (32)	16 (27)	6 (10)
Rejection	60	3 (5)	36 (60)	21 (35)	0

^{B/} "partially chilled"^{B/} After 4 days at 20°C

Table 5

Vibrio isolated from mullet, bream and bay trout ^{B/}

PERIOD	NUMBER	<i>Aeromonas hydrophila</i>	<i>Aeromonas sobria</i>	<i>Aeromonas salmonicida</i>
Initial	19	3 (16)	1 (5)	15 (79)
Rejection	36	30 (83)	0	4 (12)

^{B/} "Partial chilling"

Table 6

Biochemical activity of VRCA isolates

Biochemical activity	Percentage of isolates ^{2/} of	
	<i>Aeromonas hydrophila</i>	<i>Enterobacteriaceae</i>
TMAO reduction	30 (100)	19 (90)
H ₂ S production	30 (100)	21 (100)
Volatile sulphide production (other than H ₂ S)	5 (17)	12 (57)

^{2/} Saccharial isolates of "partially chilled" marine fish at rejection

Table 7

Off-odours detected

Isolates	Inoculated muscle	Spoiled fish
<i>Aeromonas hydrophila</i>	ammoniacal	ammoniacal
	putrid	putrid
	pungent	pungent
	faecal - like	faecal - like
	slightly "sulphidic"	slightly "sulphidic"
<i>Enterobacteriaceae</i>	pungent	pungent
	slightly "sour"	-----
	slightly "fruity"	-----
	"atale"	-----

^{2/}Off-odours" after storages at 10°C for 4 days then at 29°C

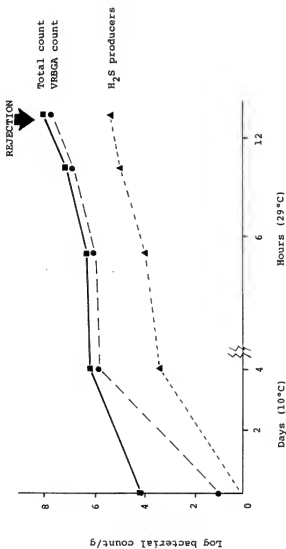


Figure 1 Microbiological changes^{1/} in "partially chilled" bream (*Acanthopagrus butcheri*)

^{1/} Similar trends were obtained on partially chilled bay trout (*Arrypis trutta*) and bream (*Acanthopagrus butcheri*)

MESOPHILIC SPOILAGE OF WHITING (*Sillago maculata*)
AND TILAPIA (*Oreochromis niloticus*)

by

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ABSTRACT

Whiting (*Sillago maculata*) stored at ambient temperature (29°C and 71% RH), spoiled within 12 h. When rejected by a trained taste panel fish had a bitter flavour and mushy texture with sour to ammoniacal odour. Tilapia (*Oreochromis niloticus*) which was rejected on the 16.5 h of ambient storage (27°C and 92% RH) had a slightly putrid, bitter and itchy flavour. No softening of the texture of the raw fish was observed. At rejection the SPC at 37°C was 10^7 - 10^8 /g; H_2S producers 10^3 - 10^4 /g; *Enterobacteriaceae* counts on VRBG, 10^6 - 10^7 /g. At rejection, chemical indices of spoilage such as K-value reached 60 and 53%; Hx concentration 2.22 and 0.8 µM/g; TMA content 13 mg% and TVN value 52 and 15 mg% for whiting and tilapia, respectively.

1. INTRODUCTION

Fish caught by the Philippine municipal fishermen which comprise 56.2% of the total fish catch (BFAR, 1982) are seldom iced or not iced at all. Explanations for this practice are because ice is very expensive and not readily available in rural communities.

While much work has been done on iced tropical fish, there are just a few studies done on fish kept at ambient temperature, which characterize fishing operations in tropical countries like the Philippines (Reilly, Bernarte and Dangla, and Barila *et al.*, these proceedings). Knowledge of the mechanism of fish spoilage at ambient temperature and the maximum time that the fish can be kept at this temperature and still be of good quality will be a great help to the fishing industry in the tropics.

This study investigated the spoilage patterns of a marine and freshwater fish stored at ambient temperatures.

2. METHODS

Four storage trials on whiting (*Sillago maculata*) locally known as asohos caught in Manila Bay along the shores of Cavite were carried out. Small fishing boats which could carry a maximum of three people were used to catch the fish. Two storage trials were carried out on tilapia (*Oreochromis niloticus*) a freshwater fish from ponds in Nueva Ecija. The fish were brought to the laboratory, live. The fish were ungutted and left uniced and covered with screens to protect fish from flies. The temperature and humidity profiles were monitored using a thermohygraph.

2.1 Sampling

Twenty eight (28) pieces of whiting (18-25 cm; 38-60 g) were taken for analyses at 3 h interval until fish were rejected; ten (10) for microbiological and chemical tests and eighteen (18) for sensory evaluation (Figure 1). Raw and cooked samples (gutted, cleaned and then steamed for 10 min were presented to each taste panel member. The same scheme was followed with 10 pieces of tilapia (22-29 cm, 218-402 g) per sampling except that the fish were steamed for 20 min.

2.2 Chemical Analysis

Total volatile bases (TVB) and trimethylamine (TMA): Trichloroacetic acid extracts were prepared from 30 g of fish flesh and TVN and TMA (except for tilapia) were determined by the Conway microdiffusion technique (Conway, 1968).

Hx content was determined on perchloric acid extracts according to the method of Burt, Murray and Stroud (1968). K-values which are expressed as the percentage ratio of Hx and inosine (HxR) to the total amount of ATP-related compounds were estimated by the method of Kobayashi and Uchiyama (1970).

2.3 Microbiological Analysis

The total viable count (TVC) and hydrogen sulphide producers were determined on peptone iron agar (Jensen and Schultz, 1980), after incubation at 20°C for 72 h and 37°C for 48 h. Glucose fermenters were determined using violet red bile glucose agar (VRBG).

2.4 Sensory Analysis

Sensory analysis was carried out by six (6) trained panelists using the scorecards for raw and cooked fish presented in Figures 2, 3 and 4. Shelf life was based on the cooked flavour of 4 as the limit of acceptability.

2.5 Statistical Analysis

Statistical analysis of the four experiments was done using Basic Statistics and Data Manipulation Pac (BSDM) using a HP85 for linear and exponential regression as prescribed by Amerine, Pangborn and Rooster (1965) and Gatchalian (1981). Correlation coefficients were tested at 1 to 0.1% levels of significance.

3. RESULTS AND DISCUSSIONS

3.1 Sensory Evaluation

The descriptive scorecards for whiting and tilapia are presented in Figures 2, 3 and 4. Regression analysis between flavour scores and storage time proved to be linear with negative correlation at 0.1% (figures 5-8).

Whiting was rejected by the taste panel within 12.5 h with the panelists describing the raw fish as having strong sour and fishy odours, soft texture with the skin being easily peeled off. Cooked fish had strong sour to ammoniacal odours, mushy texture and predominantly bitter flavour.

Rejection for tilapia was within 16.5 h. During this time, both raw and cooked odours were found to be strong fishy, rancid and sour; and flavour to be slightly putrid, bitter and itchy.

As a common observation in tropical freshwater fish, tilapia was still alive even up to 6 h after catch. Consequently, the sensory characteristics of the fish during that period were the same. All throughout the storage, no significant change in the texture of the raw fish was noted. Contrary to observations during ice storage, fish stored at ambient temperature did not show characteristic blood shot and sunken eyes at the rejection point.

3.2 Chemical Analysis

The indices of spoilage based on autolytic degradation of nucleotides in terms of % K-value and H_x are plotted against storage time (Figures 9-12). Percent K-values increased linearly with time and showed high correlation at 0.1% level. Both species of fish had K-values below 5% right after catch, and at rejection point 60% for whiting and 53% for tilapia. These rejection levels coincide with the 60% set by Japanese workers (Saito, Arai and Matsuyoshi, 1959; Ehira, 1976; Ehira and Uchiyama, 1974) who studied at least 110 species. These same authors established a 20% K-value as the freshness limit for sashimi fish. Fish kept at ambient conditions reached this value in about 4.5 and 6.8 h for whiting and tilapia respectively. Since in practice these two species are seldom iced by the fishermen/pond owners, and icing is left to the retailers, it is recommended that icing delays should not exceed these limits.

H_x values showed exponential increase with storage time significant at 0.1% level for both species under study (Figures 10 and 12). It should be noted that this trend follows that of bacterial count, which might mean that H_x formation in ambient stored fish, especially during the later part, is generally bacterial in nature. At rejection, whiting reached a level of 2.22 and tilapia 0.8 $\mu\text{M/g}$. The pronounced bitter flavour in whiting during the end of storage could be due to its high H_x content at rejection. This is in agreement with Jones' (1961) earlier report that concentrations about 2 $\mu\text{M/g}$ H_x causes bitter flavour.

TMA and TVN contents of whiting showed slow increase during the first half of storage and levels increased more rapidly during the latter stages. Exponential curve fit gave correlation coefficients which are significant at 0.1% level (Figures 13-15). During ambient storage TMA and TVN proved to be reliable quality indices unlike in ice storage where the melting ice leaches out these compounds. Shewan (1976) found bacteria to be mainly responsible for the breakdown of non-protein nitrogenous compounds to products like TMA and TVN. This close association of TMA and TVN to bacterial numbers explains the exponential behaviour of the graphs. For whiting, the TMA value of 13 mg% at the end of storage is within the 10-15 mg% limit of acceptability reported by Connell (1975) for ice stored fish but the TVN value, 52 mg% was higher than the 30-40 mg% limit. Tilapia showed lower TVN values of 15 mg%. The low TVN value for tilapia could possibly explain the not too offensive odour of the fish, even at the end of the storage, unlike whiting where the raw odour was more offensive.

3.3 Microbiological Analysis

The bacterial loads are presented in Figures 16 to 19. The initial and rejection bacterial level of the two species were within the range reported for temperate species of 10^3 - 10^6 /g (Shewan, 1976) and those for tropical fish and shellfish (Ratilly, Barnarta and Dangla and Barille, *et al.*, these proceedings). Unlike in iced storage of other tropical species, the H_2S producers or the black colonies did not become dominant (1-13%) during ambient storage. VRBCA is a medium used for the enumeration of *Enterobacteriaceae* which are all mesophiles. In this study, the *Enterobacteriaceae* (VRBC counts at 20 and at 37°C) were higher in number compared to the H_2S producers at all points during the storage of both species. These results tend to suggest that these organisms play an important role in the mesophilic spoilage of both species studied.

4. REFERENCES

- Amerina, M., R. Pangborn and E. Roastar, Principles of sensory evaluation of food. New York, 1965 Academic Press
- BFAR (Bureau of Fisheries and Aquatic Resources), Fisheries statistics of the Philippines, Manila, 1982 BFAR
- Burt, J.R., J. Murray and G.D. Stroud, An improved automated analysis of hypoxanthine. J. Food Technol., 3:165-70
- Connell, J.J., Control of fish quality. Farnham, Surrey, Fishing News (Books) Ltd., 179 p. 1975
- Conway, E.J., Microdiffusion analysis and volumetric error. London, Crosby, Lockwood and Son Ltd., 1968 465 p.
- Ehira, S., A biochemical study on the freshness of fish. Bull. Tokai Reg. Fish. Res. Lab., 88:1-128 1976
- Ehira, S. and H. Uchiyama, Freshness-lowering rates of cod and seabream viewed from changes in bacterial count, TVN and TMA-nitrogen and ATP related compounds. Bull. Jap. Soc. Sci. Fish., 40:479-88
- Gatchalian, M.M., Sensory evaluation methods with statistical analysis. Quezon City, College of 1981 Home Economics, University of the Philippines, 421 p.
- Jensen, M.H. and E. Schultz, Utilisation of iron agar in determining the freshness of wet fish. 1980 Dan. Vet. Tidsskr., 63:314-8
- Jones, N.R., Fish flavours. Torry Mem., (64):21 p. 1961
- Kobayashi, B. and H. Uchiyama, Simple and rapid method for estimating the freshness of fish. Bull. Tokai Reg. Fish. Res. Lab., 61:21-6 1970
- Saito, T., K. Arai and M. Matsuyoshi, A new method for estimating freshness of fish. Bull. Jap. Soc. Sci. Fish., 24:749-50 1959
- Shewan, J.M., The bacteriology of fresh and spoiling fish and biochemical changes induced by bacterial action. In Proceedings of the Conference on the handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 377-94

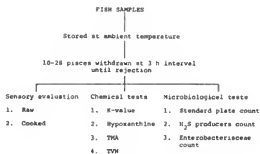


Figure 1 Sampling scheme

0	2	4	6	8	10
ODOUR					
Putrid Ammoniacal	Sulfidic Slightly putrid	Strong sour Strong fishy	Slightly fishy Slightly sour	No odour	Fresh seaweed
SKIN					
Dull yellow slime like coating all over body	Very dull -faded gray -yellowish Slime coating	Dull skin pigmen- tation -faint violet to dull gray -yellowish dull silvery tint -dull yellowish slime starts to develop -yellowish-green reddish discolor- ation	-faint violet -dull silvery tint -pale yellowish green	Shiny skin -light violet -shiny silvery tint -yellow-green	Shiny skin -violet sheen (dorsal) -glossy silvery tint (ventral) -yellowish-green fin/portion slimy lateral lines -transparent, yellowish (snout)
TEXTURE					
Mushy	Very soft	Soft	Slightly soft	Slightly firm	Firm elastic Firm elastic rigid
CONDITION OF GILLS					
	Pale (Pinkish)	Pale brown watery mucous	Red-brown	Reddish maroon	Bright red thin mucous
EYES					
Eyes starting to get loose from its socket	Eyeball beginning to loosen -opaque -translucent -sunk	-slightly sunken -slightly opaque -translucent	-flat -black -less transparent	-slightly -black pupil -transparent cornea	-convex (shag.) -bright clear black pupil -clear transparent cornea

Figure 2 Scorecard for raw whiting (*Sillago maculata*) stored at ambient temperature

0	2	4	6	8	10
ODOUR					
Strong sulfidy -Putrid/ Haussating	-Sulfidy -Ammonical	"cheasy" -strong fishy	-Slightly sour -slightly "cheasy" (fermented odour)	-Little odour to slightly fishy	slightly sweet Character- istic sweet odour "Boiled rootcrop" -sawedy
FLAVOUR					
Putrid Haussating	-Bitter -Spoiled/ sour	-Bitter -Slightly putrid	-Slightly sour -slightly bitter fishy to creamy	-Little flavour -Trace of fishy	slightly sweet -meaty Character- istic sweet flavour "Boiled potato"
TEXTURE					
Very soft and mushy		-soft to mushy	-slightly soft -slightly meaty	-slightly firm -chawy	-slightly firm and slippery -Flaky -Succulent (juicy)

Figure 3 Scorecard for cooked whiting (*Sillago maculata*) stored at ambient temperature

0	2	4	6	8	10		
RAW FISH ODOUR							
	sulfidy slightly putrid ammoniacal	-strong fishy -slightly rancid	slightly fishy	faint fishy	neutral fresh grassy		
CONDITION OF GILLS							
	pale brown	brown	pale maroon to brown thin/brown slime	maroon to brownish red thin/ yellowish slime	less bright stringy/ clear slime	bright as berry thick clear slime	
COOKED FISH ODOUR							
Ammonical sulfidy putrid	cheesy (fermented fish)	slightly rancid slightly sulfidy	boiled cassava greasy	strong rootcrop cooked prawn-like	slightly carnest- like, bland		
FLAVOUR							
	putrid ammonical	slightly putrid sour, bitter biting	canned sardines slightly cheasy slightly bitter slightly biting	blend. traces of bitter/ biting	cassava-like less crabmeat- like bland	slightly crabmeat like, bland	
TEXTURE (Mouth feel)							
		fibrous/ dry greasy	fibrous	cheasy	cheasy/less juicy/very soft	soft/more juicy/ loss of stickiness	sticky, slippery/ loss of juicy

Figure 4 Scorecard for tilapia (*Oreochromis niloticus*) stored at ambient temperature

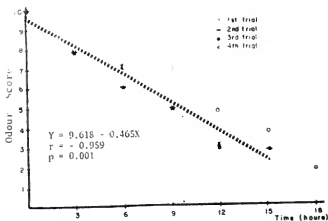


Figure 5 Odour scores of whiting (*Sillago maculata*) during storage at ambient conditions ($T = 29^{\circ}\text{C}$; $\text{RH} = 71\%$)

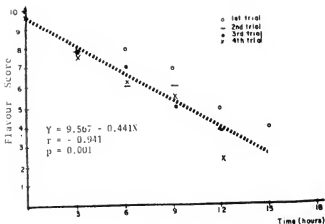


Figure 6 Flavour scores of whiting (*Sillago maculata*) during storage at ambient conditions ($T = 29^{\circ}\text{C}$; $\text{RH} = 71\%$)

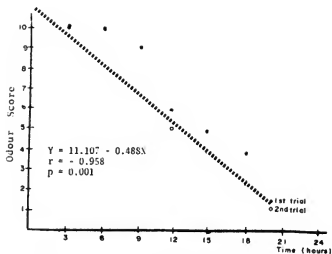


Figure 7 Odour scores of tilapia (*Oreochromis niloticus*) during storage at ambient conditions ($T = 27^{\circ}\text{C}$; $\text{RH} = 82\%$)

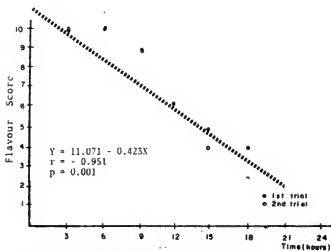


Figure 8 Flavour scores of tilapia (*Oreochromis niloticus*) during storage at ambient conditions ($T = 27^{\circ}\text{C}$; $\text{RH} = 82\%$)

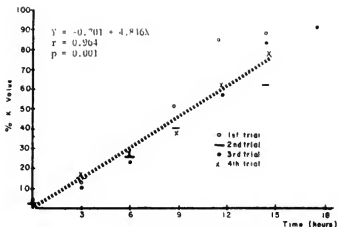


Figure 9 Changes in K-value in whiting (*Sillago maculata*) during storage at ambient conditions ($T = 29^{\circ}\text{C}$; $\text{RH} = 71\%$)

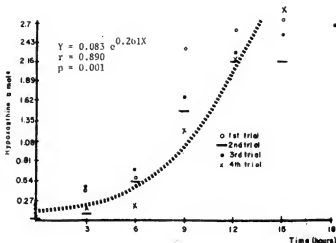


Figure 10 Changes in Hypoxanthine content in whiting (*Sillago maculata*) during storage at ambient conditions ($T = 29^{\circ}\text{C}$; $\text{RH} = 71\%$)

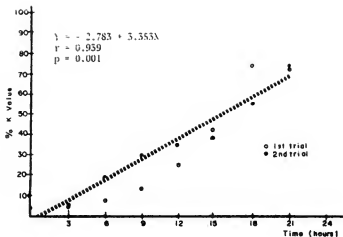


Figure 11 Changes in K-value in tilapia (*Oreochromis niloticus*) during storage at ambient conditions (T = 27°C; RH = 82%)

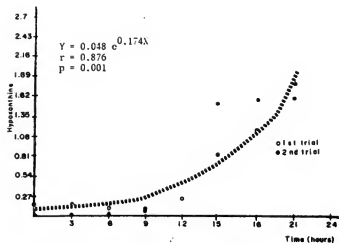


Figure 12 Changes in Hypoxanthine content in tilapia (*Oreochromis niloticus*) during storage at ambient conditions (T = 27°C; RH = 82%)

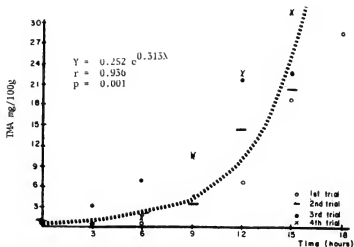


Figure 13 Changes in Trimethylamine in whiting (*Sillago maculata*) during storage at ambient conditions (T = 29°C; RH = 71%)

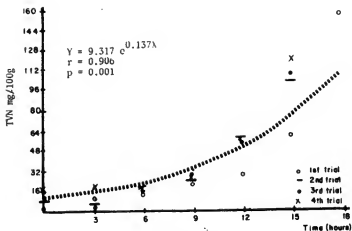


Figure 14 Changes in total volatile Nitrogen in whiting (*Sillago maculata*) during storage at ambient conditions (T = 29°C; RH = 71%)

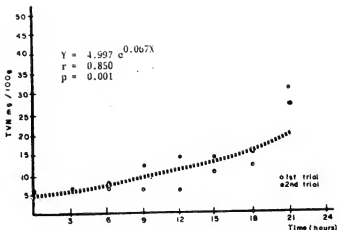


Figure 15 Changes in total volatile Nitrogen in tilapia (*Oreochromis niloticus*) during storage at ambient conditions ($T = 27^{\circ}\text{C}$; $\text{RH} = 82\%$)

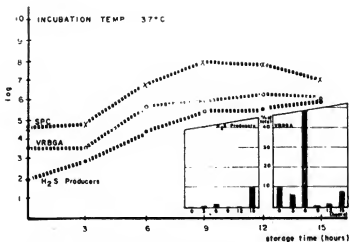


Figure 16 Changes in SPC, H_2S Producers count, VRBGA count, % H_2S Producers and % VRBGA over the total counts during storage of whiting (*Sillago maculata*) at ambient conditions ($T = 29^{\circ}\text{C}$; $\text{RH} = 71\%$)

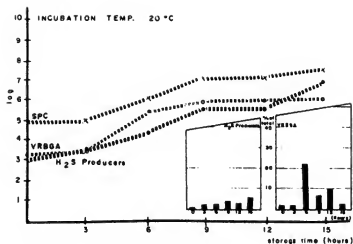


Figure 17 Changes in SPC, H₂S Producers count, VRBGA count, % H₂S Producers and % VRBGA over the total counts during storage of whiting (*Sillago maculata*) at ambient conditions (T = 29°C; RH = 71%)

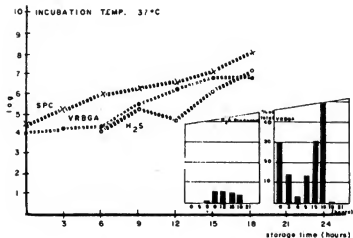


Figure 18 Changes in SPC, H₂S Producers count, VRBGA count, % H₂S Producers and % VRBGA over the total counts during storage of tilapia (*Oreochromis niloticus*) at ambient conditions (T = 27°C; RH = 82%)

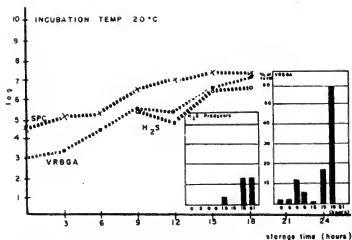


Figure 19 Changes in SPC, H₂S Producers count, VRBGA count, % H₂S Producers and % VRBGA over the total counts during storage of tilapia (*Oreochromis niloticus*) at ambient conditions (T = 27°C; RH = 82%)

SPOILAGE PATTERNS OF MACKEREL (*Rastrelliger faughni* Matsui)
2. MESOPHILIC AND PSYCHROPHILIC SPOILAGE

by

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ABSTRACT

Faughn's mackerel (*Rastrelliger faughni* Matsui) caught off the east coast of the Philippines stored for 15 days on ice compared with 14, 7 and 4 days in seawater at 0, 5 and 10°C respectively; and less than 1 day at ambient temperature (28°-30°C). The initial bacterial load was identified as predominantly mesophilic *Acinetobacter* (45%) and *Bacillus* (27%). When fish were rejected by a trained taste panel the standard plate count (20°C) was approximately 10⁷/g at all storage temperatures. The composition of the bacterial load at rejection changed at the different storage temperatures. The principal species at 0°C in ice were *Pseudomonas* spp. (50%) and *Alteromonas putrefaciens* (33%); at 0°C in seawater *Aeromonas hydrophila* (42%), *Pseudomonas* spp. (33%) and *Alteromonas putrefaciens* (17%); at 10°C *Aeromonas hydrophila* (25%), *Bacillus* spp. (17%); and at ambient temperature *Alteromonas putrefaciens* (40%), *Proteus* spp. (26%) and *Pseudomonas* spp. (20%). Nucleotide decomposition as determined by K-values were 53-76% at rejection, while hypoxanthine levels were around 1.0/μM/g. TMA and TVN concentrations served only as indicators of spoilage and did not correlate with storage time.

1. INTRODUCTION

In many rural fishing communities in developing countries fish are not iced or inadequately iced after catching. For example, in the Philippines fish are transported and stored in conical steel tubs called *baneras*. These containers are not insulated and have no provision for melt water drainage. As a result fish are stored in ice-water slurries at temperatures above 0°C, which leads to rapid quality deterioration. Storage under these conditions result in fish being unsuitable for processing and finally unfit for human consumption. While many studies exist on the storage of tropical fish on ice (Lima dos Santos, 1981), few documented investigations have been carried out on the spoilage of fish at high tropical ambient temperatures (Disney, 1976; Poulter, Curran and Disney, 1981). No data are available on how the bacterial load changes during storage under tropical commercial conditions or which species are important during spoilage.

The objective of our study was to compare spoilage characteristics of fish stored at higher temperatures with the shelf life in ice and chilled seawater (CSW) at 0°C. We have compared some of the chemical, sensory and microbiological changes that take place when Faughn's mackerel (*Rastrelliger faughni* Matsui) is stored in ice, in CSW at 0, 5 and 10°C; and at ambient temperatures (28-30°C). This study simulates commercial chilling methods where fish are stored at temperatures above 0°C during distribution and marketing.

2. MATERIALS AND METHODS

Two storage trials were carried out during these experiments with fish caught off the east coast of the Philippines. Researchers went on board commercial purse-seiners where fish were caught at night using lights or floating rafts as fish aggregating devices (Matsumoto, Kezama and Aasted, 1981; Wickham, 1973). Fish were transferred directly from the purse-seine nets to CSW at 0, 5 and 10°C. Samples at ambient temperature were held in seawater at 27-29°C, and control samples were iced with a 1:1 ratio of fish to ice. Samples for initial microbiological tests were packed in a

sterile Colworth stomacher bag, sealed and then iced while samples for proximate and chemical analyses were frozen in dry ice. All samples were kept in insulated boxes and transported back to the laboratory of the UPV College of Fisheries where analyses were carried out within 24 h after catch. Insulated boxes were stored in a cold room at 5°-8°C throughout the experiment to reduce temperature fluctuations, except for samples held at ambient temperature. Ice was added to maintain different storage temperatures and mixed twice daily to achieve a uniform temperature throughout the storage period. The temperature profiles of fish during storage are presented in Table 1.

Table 1

Internal temperature of Faughn's mackerel during chill storage

Chilling medium	Temperature range (°C)	Average temperature (°C)
Ice	0.0-1.25	1.0
0°C, CSW	0.0-1.0	0.0-1.0
5°C, CSW	4.5-6.0	5.0-5.8
10°C, CSW	7.0-10.0	10.0
28-30°C, SW (ambient)	27.0-29.0	27.0-28.0

Sensory, chemical and microbiological tests were carried out as previously described (Barile *et al.*, these proceedings).

3. RESULTS AND DISCUSSIONS

Results of the bacteriological examination are presented in (Fig. 1). Regardless of the storage temperature, fish were considered spoiled when the SPC's at 20°C reached 10^6 /g. The number of hydrogen sulphide producers varied throughout storage at different temperatures but on rejection exceeded 10^6 /g. Similar results have been reported for temperate species of fish (Liston, 1982). Storage in CSW resulted in a higher percentage of hydrogen sulphide producing bacteria, most likely as a result of contamination by gut contents after "belly bursting" and the depletion of oxygen in the chilling medium. The hydrogen sulphide producers which tolerate low temperatures are extremely important in spoilage of proteinaceous foods during chilled storage. When the number of these bacteria exceed 10^6 /g significant amounts of volatile sulphur containing compounds are produced and spoilage becomes organoleptically evident (Liston, 1982).

The changes in bacterial flora of Faughn's mackerel during storage are presented in Figure 2. The initial load was quite diverse comprising of *Acinetobacter* (45%), *Bacillus* spp. (27%), *Pseudomonas* spp. (9%) and *Alteromonas putrefaciens* (9%). During iced storage *Pseudomonas* spp. and *Alteromonas putrefaciens* increased in numbers and accounted for 83% of the spoilage flora when fish were rejected by the taste panel on day 15. The same species are active spoilers of temperate fish like cod (Herbert *et al.*, 1971; Shewan, 1977) and haddock (Levin, 1968). Both genera were present throughout storage at 0°C, CSW and accounted for 50% of the spoilage flora with *Aeromonas hydrophila* and *Bacillus* spp. comprising 42 and 8% respectively. *Aeromonas hydrophila* also predominated the bacterial load at 50% after 8 days storage at 5°C and 25% after 6 days storage at 10°C, although it was not isolated as part of the original flora. Fish stored at ambient temperature spoiled rapidly and the flora consisted of *Alteromonas putrefaciens* (40%), *Proteus* spp. (26%), *Pseudomonas* spp. (20%) and *Bacillus* (7%).

On harvesting, Faughn's mackerel had a predominantly mesophilic bacterial population. During iced storage psychrotrophs predominated the spoilage flora. This suggests that, irrespective of the original bacterial flora, spoilage during iced storage is caused by *Pseudomonas* spp. and *Alteromonas putrefaciens* similar to the spoilage patterns reported for temperate fish (Shewan, 1977; Levin, 1968; Herbert *et al.*, 1971). During storage in 0° and 5°C CSW, a third genus *Aeromonas hydrophila* constituted a major part of the spoilage flora. This is the first time this organism has been reported as a spoiler of tropical fish although it is known to cause off flavours in refrigerated dairy products

(Law et al., 1979). The most likely source of *Aeromonas hydrophila* is contaminated ice, as we have found it present as part of the bacterial flora of ice from a number of local ice plants. At the higher storage temperatures, no single species dominated the spoilage flora.

Proximate composition of Faughn's mackerel used in this experiment is presented in Table 2.

Table 2

Proximate composition of Faughn's mackerel

Fish part	% Moisture	% Protein	% Fat	% Ash
Whole	74.6	14.31	1.95	2.52
	73.5	13.68	2.54	3.05
Flash	75.1	17.08	0.74	0.76
	75.5	16.15	0.45	1.24

Linear regression of cooked flavour scores and storage time are presented in Fig. 3. Samples were rejected after 15, 14, 7, 4 days and less than 1 day storage in ice; 0°C, 5°C, 10°C, CSW and ambient temperature respectively. When spoiled, fish had bitter, sour, rancid flavours with an "itchy" after-taste and sulphidic, fishy odours. The texture was dry and fibrous. Fish stored at ambient temperature were rejected on the basis of raw odour and texture which were putrid/ammoniacal and very soft flesh. Fish stored at 0°C in CSW showed better quality than ice stored fish up to 10 days, however from the 10th day until the end of storage, these samples deteriorated more rapidly than the iced fish. Similar results were found for sardines stored in chilled seawater (Barhoumi et al., 1981). The principal change in quality of Faughn's mackerel stored in CSW at and above 0°C was the development of a sour odour and flavour which became more pronounced approaching rejection. Iced fish, on the other hand showed a gradual loss of sweet characteristic flavour and odour, with the development of a bland taste and finally sour, fishy, rancid flavours.

The incidence of "belly bursting" increased with increasing storage temperature (Fig. 4). This defect was lowest in the iced samples and reached 80 and 90% during storage at 0°C and 5°C/10°C CSW respectively.

The changes in % K-values during storage at different temperatures with corresponding regression equations are shown in Fig. 5. The K-value of fish immediately after landing was 5%. This increased with storage time, the rate of increase being faster at higher temperatures. At rejection % K-values were 58, 80, 58 and 51% for fish stored in ice; 0°C, 5°C and 10°C CSW respectively.

K-values have been suggested as a freshness index for fish by many authors (Ehire, 1976; Saito, Arai and Matsuyoshi, 1959; Ehire and Uchiyama, 1974). In our study regression analysis showed high correlation between K-values and storage time at 1% level for all temperatures. The rejection level of 51-80% based on cooked flavour scores agree with the rejection limit of 60% set by (Ehire, 1976). K-value rejection level based on cooked flavour scores is close to the rejection limit of 60% set by Ehire (1976), except for fish stored at 0°C CSW which gave a higher value of 80%. On the other hand, the same author suggested a K-value of 20% as a freshness limit for fish. Iced stored Faughn's mackerel reached this value within 4 days, after 2.5 days for 0°C, CSW, after 1.5 days at 5°C, CSW and 24 h at 10°C CSW. Fish should reach the consumers or processors within these time limits if it is to be utilized in a very fresh condition.

Hypoxanthine has been proposed as a measure of fish freshness for some species (Jones, Murray and Burt, 1965). The limit of acceptability for Faughn's mackerel was in the range 1.0-1.1 µM/g. The increase in Hx concentration with storage are shown in Fig. 6. The results are similar to % K-values in that the rate of Hx formation was fastest at higher storage temperatures and correlated significantly with storage time. When fish were rejected the final concentration in µM/g were 1.07, 1.08, 1.00 and 0.99 for fish stored in ice; 0°C, 5°C and 10°C, CSW respectively. These values are similar to those reported for red snapper (Boyd and Wilson, 1977) but lower than chub mackerel (Poulter, Curran and Disney 1981) and seabream (Curran et al., 1980). Different fish species can accumulate Hx of HxR during chill storage (Ehire, 1976). In a related study Faughn's mackerel was found to accumulate inosine (HxR) when stored in ice (Estrade, 1983).

The TMA and TVN results are shown in Table 3. When fish spoiled TMA values ranged from 1.44-1.82 mg/100 g and TVN 18.05-21.29 mg/100 g for all storage temperatures. The values fluctuated during storage and showed no significant correlation with storage time and sensory results. Both TMA and TVN are of questionable use as quality indices of some tropical fish (Amu and Disney, 1973; Lima de Santos, 1981). Our results tend to confirm these observations as values varied throughout storage and the limit of acceptability, 10-15 mg TMA/100 g (Connell, 1975) were never approached.

Table 3

Changes in TMA and TVN (mg/100 g) values during storage at different temperatures

Days	Ice		0°C, CSW		5°C, CSW		10°C, CSW	
	TMA	TVN	TMA	TVN	TMA	TVN	TMA	TVN
1	0.27	19.56	0.61	15.28	0.17	17.67	0.61	17.67
3	0.24	15.15	0.30	15.15	0.45	14.53	0.76	15.47
6	0.45	19.24	0.45	13.27	0.91	14.84	1.44	20.63
8	0.83	18.93	0.82	11.82	1.82	19.56		
10	0.83	21.38	0.98	18.05				
13	1.12	20.75	1.82	18.05				
15	1.82	21.29						

4. CONCLUSION

Our study emphasises the importance of rapid chilling and storage of fish at low temperatures in the tropics. Overall results based on sensory, microbiological and chemical analyses show that, for every 5°C above 0°C fish are stored, a 50% reduction in shelf life occurs.

Taste panel results correlated significantly with storage time, K-values and Hx concentration for all storage temperatures. Storing fish at 0°C in CSW results in better keeping quality than iced storage for up to 10 days. However, the overall storage life is similar indicating that the method of chilling is of minor importance, providing the fish are maintained at 0°C. The sour odour observed in CSW stored fish can be attributed to the higher percentage of hydrogen sulphide producing bacteria which developed rapidly when compared to ice stored fish. Storage at 5°C, 10°C and ambient temperature leads to a dramatic loss of quality and a substantial reduction of keeping time. When compared with spoilage at ambient tropical temperatures there are considerable advantages in storing fish in seawater at or below 10°C. When ice is expensive or not readily available to maintain the fish at 0°C, the shelf life is sufficiently extended to allow transport to retail markets.

Sensory analysis by trained taste panels is probably the most important method of assessing fish quality. In developing countries, particularly in rural fishing communities, facilities are generally available to carry out chemical or microbiological testing and acceptability is determined solely by sensory attributes.

5. REFERENCES

- Amu, L. and J.C. Disney. Quality changes in West African marine fish during iced storage. Trop. Sci., 15:125-40
- Barhoumi, M., et al., Storage characteristics of sardines (*Sardinella pilchardus* Welbeum) held in ice and chilled seawater. In Advances in the technology of chilling, freezing, processing, storage and transport of fish, especially underutilised species. Sci.Tech.Froid/Refrig.Sci.Technol.Int.Inst.Refrig., (1981-4):243-9
- Boyd, N.S. and N.D.C. Wilson. Hypoxanthine concentrations as an indicator of freshness of iced snapper. N.Z.J.Sci., 20:139-43
- Connell, J.J., Control of fish quality. Fernham, Surrey, England, Fishing News (Books) Ltd., 179 p. 1975
- Curran, G.A., et al., Spoilage of fish from Hong Kong at different storage temperatures. 1. Quality changes in gold-lined seabream (*Rhalaeargus sarba*) during storage at 0°C (in ice) and 10°C. Trop.Sci., 22:367-82

- Disney, J.G., The spoilage of fish in the tropics. In Proceedings of the First Annual tropical and subtropical Fisheries Technological and Conferanca. Collaga Station, Texas A+M University, vol.1:23-39
- 1976
- Ehira, S., A biochemical study on the freshness of fish. Bull.Tokai Reg.Fish.Res.Lab., (86) 1976
- Ehira, S. end M. Uchiyama, Freshness-lowering retes of cod end seebream viewed from changes in bacterial coun, TVB and TMA-Nitrogen and ATP related compounds. Bull.Jap.Soc.Sci.Fish., 40:479-87
- 1974
- Estrada, M.B., Spoilage pectern of *alimchan* (*Rastrelliger fanghni* Matsui) stored in ica. M.S. Thesis, Collaga of Homa Economics, University of the Philippines, 148 p.
- 1983
- Harbert, P.A., et al., Bectaria active in the spoilage of certain sea foods. J.Appl.Bacter., 31:41-50
- 1971
- Jones, N.R., J. Murray and J.R. Burt, Automated analysis of hypoxanthine. J.Food Sci., 30:791-4
- 1965
- Lew, B.A., et al., Psychotropha end their effects on milk end spoilage and the environment, edited by A.D. Russell and R. Fuller. London, Academic Press, pp. 137-50
- 1979
- Lavin, R.E., Detection end incidence of specific species of spoilaga bacteria of fish. Appl. Microbiol., 16:1734-7
- 1968
- Lima dos Santos, C.A.M., Tha storage of tropical fish in ice - a ravier. Trop.Sci., 23:97-127
- 1981
- Liston, J., Recent advances in the chemistry of iced fish spoilage. In Chemistry and biochemistry of marina products, edited by R.E. Martin, et al. Westport, Connecticut, AVI Publishing Co., pp. 27-38
- 1982
- Matsumoto, W.M., T.K. Kezama and D.C. Austed, Anchored fish eggregeting devices in Hawaiian waters. Mar.Fish.Rev., 43:1-13
- 1981
- Poulter, R.G., C.A. Curran and J.G. Disney, Chill storega of tropical and temperate water fish - differences and similarities. In Advances in technology in chilling, freezing, processing, storage and transport of fish, especially underutilised species. Sci.Tech.Froid/Refrig.Sci.Technol.Int.Inst.Refrig., (1981-4):111-22
- 1981
- Poulter, R.G., L. Nicolaidas end D.A. Hector, Quality changes in fish from the South China Sea: iced storage of chub mackerel, grouper and fusilier. Proc.IPFC, 18(3):169-85
- 1978
- Saito, T., K. Arai and M. Matsuyoehi, A new method for estimating the freshness of fish. Bull.Jap.Soc.Sci.Fish., 24:7
- 1959
- Shewan, J.M., The bacteriology of fraeh end spoiling fish end biochemical changes induced by bacterial action. In Procmaings of the Conference on the handling, processing, marketing of tropical fish. London, Tropical Products Institute, pp. 51-66
- 1977
- Wickham, D.A., Attracting and controlling coastal pelagic fish with night lights. Trans.Am.Fish.Soc., 102:816-25
- 1973

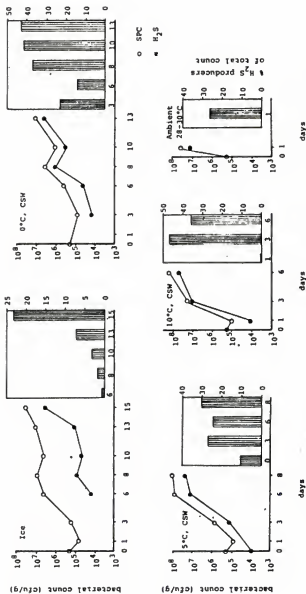


Figure 1 Standard plate count at 200C, H₂S producers count at 200C and % H₂S producers of Faughn's mackerel during storage at different temperatures

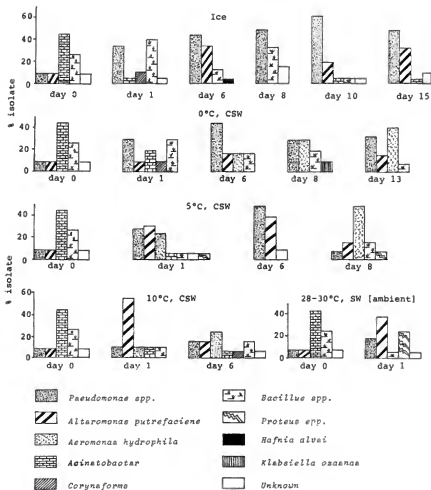


Figure 2 Changes in bacterial flora (%) of Faughn's mackerel during chill storage at different temperatures. Ice, CSW at 0, 5, 10 and ambient (28°-30°C)

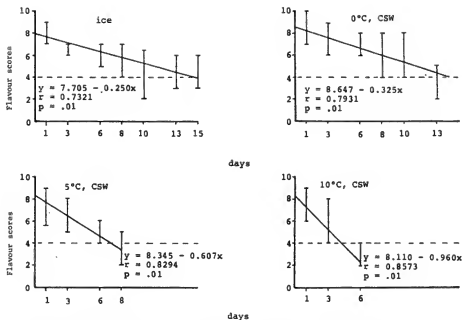


Figure 3 Correlation between cooked flavour score and storage at different temperatures

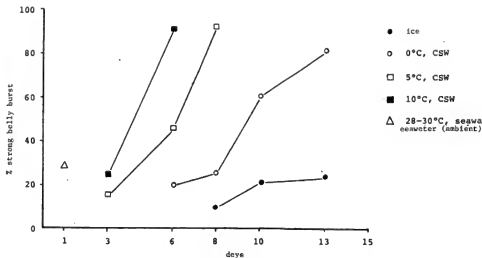


Figure 4 Incidence of belly burst (%) in Feughn's mackerel during storage at different temperatures

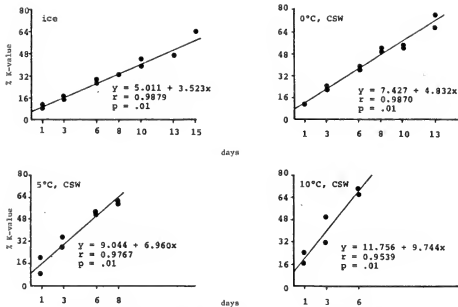


Figure 5 Correlation between % K-value and days storage at different temperatures

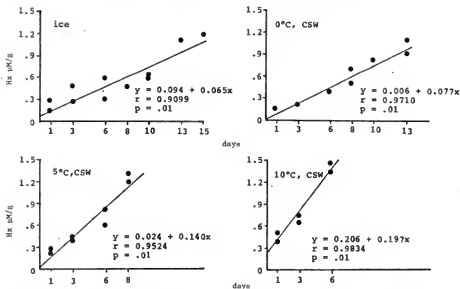


Figure 6 Correlation between hypoxanthine (Hx) and days storage at different temperatures

FROZEN STORAGE LIFE OF TROPICAL FISH SPECIES

by

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ABSTRACT

An increasing number of tropical countries are installing freezing facilities for fish, either for local distribution and consumption or to export the frozen products. For both purposes it is important to know for how long fish can be stored without significant loss in quality. However, most of the scientific work on the quality of frozen fish relates to cold-water species, and not much is known about the keeping quality of tropical species. This paper reviews the published literature on the length of time for which particular species of fish can be stored without loss of quality, at temperatures from -50° to -30°C , and the changes which occur during storage. For the more fatty species, the onset of rancidity limits the storage life. For the less fatty species, the development of a tough texture is the limiting process.

1. INTRODUCTION

The shelf life in melting ice of commercially important cold- and temperate-water species of fish has been studied in considerable depth, particularly in Europe, Japan and North America. Before the early 1970s, with the notable exception of the results of some very interesting Indian studies, there was very little information on the storage life of iced tropical species. This fact was drawn to the attention of research workers at a number of international meetings organized by FAO, the Tropical Products Institute, etc., and in the subsequent years, with the assistance of such bodies as the IPFC Working Party on Fish Technology, many more studies were carried out. This allowed the publication of two significant reviews on the subject (Curran and Disney, 1979; Lima dos Santos, 1981), which summarized the state of knowledge at that time. It was apparent, however, that even though significant advances had been made, there were still many questions unanswered and it is hoped that some of these will be covered in the papers presented at this meeting.

Whereas the use of ice may extend the storage life of fish to several weeks, freezing is an effective way of preserving fish for long periods. For freezing, unlike smoking or drying, the appearance, flavour and texture remain very similar to those of the fresh fish. After a period of frozen storage, however, the flavour and/or the texture do deteriorate, and the time it takes for this to happen is an important consideration in commercial fish-freezing operations. Many developing countries are setting up fish-freezing facilities, either for local distribution or to export the products. However, as in the early 1970s, for iced fish relates most of the work published on the frozen storage of fish to the species commonly used in the more developed countries, which come from cool or temperate waters. This paper is a first attempt to review the work which has been done so far on the frozen storage life of fish species from tropical waters. It is hoped that research workers will draw to the attention of the meeting other studies of which they are aware, and that the paper will stimulate further studies.

2. SPOILAGE OF FROZEN FISH

Research on fish from temperate waters has shown that there are four main processes involved in deterioration during storage:

- (i) Oxidation of the lipids present in the fish, resulting in rancidity (Slavin, 1968).
- (ii) Bacterial growth on the fish tissues, breaking down the structure of the tissues and causing the formation of trimethylamine and other volatile substances, which affect the smell and taste of the fish (Regenstein, *et al.*, 1982).
- (iii) Enzymic processes within the fish muscle, including the formation of hypoxanthine, which imparts a bitter flavour to the fish (Jones, 1965).

- (iv) Changes in the protein in the fish muscle, affecting the texture and causing the fish to become fibrous and chewy (Connell, 1968).

Of these, bacterial growth is important in ice storage, but at temperatures below 0°C, it is reduced and usually arrested by -10°C (Potter, 1978).

The basic method of assessing the quality of stored fish is by sensory assessment: the smell, taste, chewiness, etc. These are subjective qualities, and numerous analytical procedures have been developed to measure the amounts of particular substances in the fish in attempts to establish an objective means of assessing fish quality. However, as Gould and Peters (1980) pointed out, the accuracy of these objective tests is judged by comparing them with taste panel results. Sensory assessment is still the primary means of assessing the quality and storage life of fish.

Most of the published work on the frozen storage of tropical fish relates to pelagic fish such as sardines, various types of mackerel or tuna. It is convenient to consider these groups.

2.1 Sardines

A number of papers have described the frozen storage of *Sardinella longirostris* caught off India. The main factor affecting the storage life is the onset of rancidity (Mathen, Choudhuri and Pillai, 1966), and the time for which the fish can be stored before becoming unacceptable is inversely proportional to the oil content (Kaimal, 1969). At -18°C, the storage life ranges from 20 to 40 weeks for fish having an oil content from 42% to 102%. Glazing the fish increases the storage life (Bose, 1969), no doubt by providing a physical barrier between the fish tissues and atmospheric oxygen. This factor is well known for temperate water fish (Stansby, 1982). The use of antioxidants has been studied by Pever and Mager (1966) and Mathen, Choudhuri and Pillai (1966), but as for fish from temperate waters (Stansby, 1982), this seems to have little effect on the storage life.

Storing the fish in ice before freezing reduces the subsequent storage life. Water-glazed sardines can be stored for up to 20 weeks at -12° or -23°C (Bose, 1969; Kaimal, 1969; Shenoy and Pillai, 1971), but this period falls progressively if the fish have been stored in ice for a few days before freezing (Bose, 1969). With four days in ice, freezing, and then storage at -12° or -23°C, the fish become unacceptable after six and seven weeks, respectively. Kaimal (1969) also found a progressive shortening of the storage life of sardines stored at -23°C, but it fell from 20 weeks for fish frozen quickly to only two weeks for fish stored in ice for five days before freezing. The same effect was recorded by Shenoy and Pillai (1971), although the sardines they used became unacceptable after three days in ice. The effect is probably part of the general phenomenon observed for temperate fish species, that the frozen storage life depends on the quality before freezing (Dyer, 1968).

It is not clear whether the method of freezing affects the storage life of Indian oil sardines. Shenoy and Pillai (1971) found no difference in the storage life between fish frozen individually or in blocks. On the other hand, Bose (1969) reported differences in the protein denaturation: the protein solubility dropped more quickly for individually frozen sardines than for block frozen fish. Also, Pawar and Nagar (1966) found that the ratio of ribose not precipitated by barium acetate to total ribose was higher for fish frozen slowly than for those frozen quickly. Both these factors indicate that there should be a difference in the storage life.

2.2 Mackerel

As for sardines, most of the work on tropical types of mackerel concerns species from Indian waters.

Rastrelliger kanagurta is often known as Indian mackerel. Bose (1969) reported that glazed blocks of the species had a shelf life of 16 weeks at -20°C before rancid flavour and a hard texture developed. Sreenivasan et al. (1976) found that water-glazed *R. kanagurta* with a medium or high fat content (5-9%) became rancid and tough after four months at -20°C, whereas low-fat fish (1.8%) became texturally unacceptable after six months at that temperature. Evidently rancidity is an important cause of quality deterioration for the fatty fish, whereas textural changes become the deciding factor in low-fat fish. The use of antioxidants extended the storage life of the fatty fish to 9-10 months at -20°C (Sreenivasan et al., 1976). Similar results were reported by Jedhev and Mahar (1970).

King and Poulter (1984) examined *R. kanagurta* caught off the Seychelles Islands. Fish with lipid contents of 3-5% had a shelf life of about six months at -14°C and 16 months at -30°C. The percentage of soluble protein remained high for six months at -14°C, but then fell steadily; at -30°C there was no substantial drop during the 20 months of the trials. Neir, Copakumar and Nair (1976) described hydrolytic changes in *R. kanagurta* during frozen storage.

1/ All oil contents mentioned in this paper are expressed on a wet weight basis

Two types of chuh neckerel have been studied. *Rastrelliger neglectus* from Thailand was stored at -14° and -30°C (Key, Rettgepool and Hardy, 1972), unglazed, glazed and vacuum packed. At -14°C unglazed samples underwent substantial deterioration, but there were discrepancies between the indices used. At -14°C for glazed samples the change was small, and for vacuum packed samples only negligible changes were observed. At -30°C there was very little change over three months for any of the forms.

Poulter (1978) examined *R. brachysoma* from the South China Sea. At -10°C the fish remained in prime condition for three months and they were just acceptable after nine months. At -30°C the storage life was at least 12 months. The rate of freezing had no significant effect on the storage life. The lipid content of the fish was low, 1-3%, but the changes in peroxide value indicated that lipid oxidation was the major factor influencing the storage life. The percentage of soluble protein nitrogen showed no large decrease during the storage trials.

As for the other neckerel species - *Caranx melampygus* - a jack mackerel, stored better glazed than unglazed, 12 and 8 weeks respectively, at -18°C (Bose, 1969). The rate of freezing did not affect the storage life.

Bose (1969) also reported that seer (Spanish mackerel), *Scomberomorus commersoni*, whether frozen quickly or slowly, had a shelf life of 10 weeks at -23°C . If the fish were stored in ice for up to five days before freezing, the shelf life fell steadily to six weeks. Rancidity is again the main factor in the storage life. Hiremath and Sreenivasan (1979) found that glazed fillets of seer remained acceptable for three months at -20°C , whereas fillets treated with antioxidants before freezing retained the original flavour for 9 or 10 months.

According to Shenoy (1976), the related species *Scomberomorus guttatus* (spotted seer) had a long storage life: glazed fillets kept for 16-20 weeks at -10°C and 28-32 weeks at -23°C . Once more, keeping the fish in ice before freezing reduced the storage life: fillets kept in ice for seven days, frozen and glazed, then stored at -23°C were acceptable for only 16 weeks.

There seems to be a marked difference in the storage life of these two *Scomberomorus* species. This could be due to a difference in the fat content of the samples studied, but unfortunately, neither Bose (1969) nor Shenoy (1976) recorded the fat content of their fish. Hiremath and Sreenivasan (1979) found 10.1% of fat in his batch of the *S. commersoni* fillets.

2.3 Tuna

The freezing of tuna is commercially important as the fish are usually stored at sea for some time before canning. Nishimoto (1963) stored skipjack (species not specified, but presumably *Katsuwonus pelamis*) at -4° , -17° and -25°C . The taste panel assessment, protein extractability and histological examination indicated as storage life of one to two, 6 and 12 months, respectively. Ohta *et al.* (1967) reported that when tuna (species not stated) was stored frozen, there was a considerable drop in the protein and inosinic acid contents, but despite this, fish canned after four months storage was organoleptically indistinguishable from the control.

A problem which arises during the storage of tuna for canning is discoloration. The colour develops inside the blocks of flesh (Bito, 1964 and 1965), and is due to the oxidative change of myoglobin to metamyoglobin (Bito, 1964). Tuna stored at -5° and -10°C rapidly turns brown; at -20°C the change is slower, taking up to two months, and at -35°C the colour remains good for at least 9-13 months (Bito, 1965). The discoloration is delayed if the fish is frozen at temperatures between -10° and -40°C , immersed in liquid nitrogen, and then stored at -20°C (Bito, 1969).

2.4 Other Species

Swordfish discolour during frozen storage, but in a different way from tuna. A green colour develops beneath the skin on the ventral and lateral sides, usually in the presence of blood in the dark portions of the flesh. Below -15°C the colour develops during two weeks, but then there is no further change (Amano and Tomiye, 1953). The colour is due to sulphaemoglobin, a green compound produced by the combination of hydrogen sulphide and haemoglobin in the presence of oxygen (Teuchiye and Tatsukawa, 1954). Dyer (1969) found that swordfish fillets could be stored for three months at -8°C , nine months at -18°C and several years at -26°C . The loss of inosine monophosphate was slightly slower than the decrease in the taste panel score.

Shark flesh, mostly from *Galeus glaucus* and *Isurus nasus* discolours and develops off odours after 30 days at temperatures above -10°C (Bruno, 1953).

Among the many species studied by Bose (1969) were two species of marlin. For blue marlin, *Makaira nigricans*, unglazed fillets could be stored for 12 weeks at -20°C , glazed fillets for 16 weeks. White marlin (*M. indica*) kept for eight weeks, glazed or unglazed. For both species the main change was the development of a fibrous texture. Bombay duck, *Harpadon nehereus*, frozen at -40°C and then stored at -10°C becomes unacceptable after 12-16 weeks (Radhakrishnan, Solanki

and Venkataraman, 1973); the soluble protein nitrogen drops steadily and there is a 24% drip loss in 8-10 weeks.

One of the few non-fatty fish studied is *Priacanthus hamrus*, bigeye (King and Poulter, 1984). The fish had a lipid content of less than 0.7%. There was no drop in the soluble protein nitrogen for 63 weeks at either -14° or -30°C; after 88 weeks the SPN dropped only to 55% at -30°C and 27% at -14°C. After 88 weeks the taste panel found the fish stored at -14°C to be just acceptable, while those at -30°C were still of good quality.

The present authors are conducting frozen storage trials on two *Pristigaster* species from Vanuatu, *P. flavipinnis* and *P. multidentatus*. Unfortunately, the fish were mishandled during the shipment to London, but despite this they are showing long storage lives. The fish have a rather bland flavour and no change in this has been observed after five months' storage. The texture started to deteriorate after about four months at -5°C and five months at -10°C. These species had lipid contents below 1%.

Finally, Shenoy and Jones (1972) found a difference in the storage life of tilapia grown in fresh and brackish waters. Fish from fresh water, which were kept in ice for 13 days and then frozen, remained acceptable for 24 weeks, whereas fish from brackish water, iced for 10 days and then frozen, kept for only eight weeks.

The species studied in frozen storage trials are summarized in Table 1.

3. CONCLUSIONS

In most of the work described above, the authors have depended on sensory assessments to decide storage life. In a few cases, measurements of total volatile bases and trimethylamine have been reported, but these off-odours do not seem to have been a major factor in the storage life. Presumably, either the temperatures have been low enough to suppress bacterial growth or rancidity has developed more quickly.

Much of the work described above was done before the hypoxanthine assay (Jones, 1965) was developed, so few of the authors used this substance as an indicator of quality. Even so, most of the earlier work reports the production of rancid rather than bitter flavours.

Most of the studies have been concerned with species of fatty fish, and for these rancidity is clearly the factor limiting the storage life. Glazing extends the storage life clearly by preventing or retarding access of atmospheric oxygen to the fish tissues.

For the low fat samples of Indian mackerel (Sreenivasan *et al.*, 1976) and for bigeye (King and Poulter, 1984) rancidity is not a problem. The storage life is long or very long, and it is limited by changes in texture.

The work described above does not give a definitive indication of the storage life of the species concerned. In most papers it is clear that the fish referred to were caught in one area at a time. In the other papers the same appears to be the case. Such samples may or may not be typical of the species, since there can be large variations in composition in different areas and at different times of the year. Water temperature, food supply, breeding cycle, the size of the fish and the way in which they were killed affect the composition. For temperate fish species all these factors influence the frozen storage life (Dyer, 1968).

Consequently, it is very difficult to generalize about the frozen storage behaviour of tropical species or to compare it with that of fish from temperate waters. For storage in ice, it is generally accepted that tropical species can be stored for longer time than temperate species (Disney, Cole and Jones, 1974; Lima dos Santos, 1981). This has been related to bacteriological factors. Since deterioration during frozen storage seems to be due to rancidity or textural changes rather than bacteriological growth, this difference need not apply to frozen storage. Nevertheless, there are indications (Poulter, 1978; King and Poulter, 1984) that tropical species might have a longer frozen storage life than temperate ones. More work is needed on the frozen storage of tropical fish to clarify this point.

As we have shown, only a limited amount of work has been done on the frozen storage life of a few of the many tropical fish species. Further work would extend the existing body of knowledge about the storage of species from cooler waters, and it might help to clarify the biochemical processes controlling the quality of frozen stored fish. Such work would also provide a sound basis for the commercial use of freezing in fish production in tropical regions.

4. REFERENCES

- AMANO, K. and F. Tomiya, Studies on the green discolouration of frozen swordfish. Bull. Jap. Soc. Sci. Fish., 19(5):671 (World Fish. Abstr., 1955 May/June 33)

- Bito, M., Studies on the retention of meat colour by frozen tuna. 1. Absorption spectra of the aqueous extract of frozen tuna meat. Bull.Jap.Soc.Sci.Fish., 30(10):847-57 (World Fish. Abstr., 1967 18(1):37)
- 1965, Studies on the retention of meat colour by frozen tuna. 2. Effect of storage temperature on preventing discolouration of tuna meat during freezing storage. Bull.Jap. Soc.Sci.Fish., 31(7):534-9 (World Fish.Abstr., 1967 18(1):27)
- 1969, Studies on the retention of meat colour by frozen tuna. 7. Effect of freezing at super-low temperatures. Bull.Jap.Soc.Sci.Fish., 35(12):1193-200 (World Fish.Abstr., 1970 21(3):35)
- Boee, A.N., Freezing of tropical fish. In Freezing and irradiation of fish, edited by R. Kreuzer. Surrey, England, Fishing News (Books) Ltd., for FAO, pp. 179-88
- Bruno, G., Degradation of trimethylamine oxide and the ammonia smell of frozen shark flesh. Riv. Med.Vet.Zootec., 5:49 (World Fish.Abstr., 1955 March/April 25)
- Connell, J.J., The effect of freezing and frozen storage on the proteins of fish muscle. In Low temperature biology of foodstuffs, edited by J. Hawthorne and E.J. Rolfe. Oxford, Pergamon Press, pp. 333-58
- Curran, C.A. and J.G. Disney, The iced storage life of tropical fish. Paper presented at the IPPC 1979 Workshop on Fish Technology, Jakarta (mimeo)
- Disney, J.G., R.C. Cole and M.R. Jones, Considerations in the use of tropical fish species. In Fishery products, edited by R. Kreuzer. Farnham, Surrey, England, Fishing News (Books) Ltd., for FAO, pp. 329-37
- Dyer, W.J., Deterioration and storage life of frozen fish. In Low temperature biology of foodstuffs, edited by J. Hawthorne and E.J. Rolfe. Oxford, Pergamon Press, pp. 429-47
- 1969, Nucleotide degradation in frozen swordfish muscle. J.Fish.Res.Board Can., 26(6): 1597-1603
- Gould, E. and J.A. Peters, On testing the freshness of frozen fish. Farnham, Surrey, England, 1980 Fishing News (Books) Ltd., 2nd ed.
- Hiremath, G.C. and N. Sreenivasan, Studies on the prevention of quality loss in frozen seer fillets during storage by the use of additives. Mysore J.Agric.Sci., 13:88-92
- Jadhav, M.G. and N.Q. Maher, Preservation of fish by freezing and glazing. Fish.Technol.Soc.Fish. Technol.,Ernakulam, 7(2):146-9
- Jones, N.R., Hypoxanthine and other purine-containing fractions in fish muscle as indices of freshness. In The technology of fish utilisation, edited by R. Kreuzer. Surrey, England, Fishing News (Books) Ltd., for FAO, pp. 179-83
- Kaimal, P.N.R., Freezing of oil sardines. Indian Seafood J., 7(2):24-40 (World Fish.Abstr., 1970 1969 21(3):29)
- Keay, J.N., P. Rattagool and R. Hardy, Chub mackerel of Thailand: a short study of its chemical composition, cold storage and canning properties. J.Sci.Food Agric., 23:1359-68
- King, D.R. and R.G. Poulter, Frozen storage of Indian mackerel, *Rastrelliger kanagurta*, and big-eye, *Prionothus hamur.* Trop.Sci., submitted for publication
- Lima dos Santos, C.A.M., The storage of tropical fish in ice - A review. Trop.Sci., 23:97-127 1981
- Mathen, C., D.H. Chaudhuri and V.K. Pillai, The use of different glasses in frozen oil sardines. 1966 Fish.Technol.Soc.Fish.Technol.,Ernakulam, 3(1):30-7
- Nair, P.G.V., K. Gopakumar and M.R. Nair, Lipid hydrolysis in mackerel *Rastrelliger kanagurta* during frozen storage. Fish.Technol.Soc.Fish.Technol.,Ernakulam, 13(2):111-4
- Nishimoto, J., Studies on the change in frozen skipjack muscle during storage. Refrigeration, 1963 Tokyo, 38(424):1-5 (World Fish.Abstr., 1964 15(1):25)
- Ohta, F., et al., Frozen of skipjack prior to processing. Refrigeration, Tokyo, 42(471):14-33 1967 (World Fish.Abstr., 1968 19(3):41)

- Pewer, S.S. and N.G. Mager, Chemical changes during frozen storage of pomfret, mackerel and sardines.
1966 J.Food Sci., 31:87-93
- Potter, N.H., Food science. Westport, Connecticut, AVI Publishing Co., 232 p. 3rd ed.
1978
- Foulter, R.G., Quality changes in fish from the South China Sea. 2. Frozen storage of chub
1978 mackerel. Proc.IPF, 18(3):330-9
- Redhakrishnan, A.G., K.K. Solenki and R. Venketaraman, Freezing characteristics of Bombay duck.
1973 Fish Technol.Soc.Fish.Technol.,Ernakulam, 10(2):124-31
- Regenstein, J.M., et al., Chemical changes of trimethylamine oxide during fresh and frozen storage
1982 of fish. In Chemistry and biochemistry of marine food products, edited by R.E. Martin,
et al., Westport, Connecticut, AVI Publishing Co., pp. 137-48
- Shenoy, A.V., Freezing characteristics of tropical fishes. 3. Spotted seer. Fish.Technol.Soc.
1976 Fish.Technol.,Ernakulam, 13(2):105-10
- Shenoy, A.V. and M.A. Jones, Freezing characteristics of tropical fishes. 2. Tilepis. Fish.
1972 Technol.Soc.Fish.Technol.,Ernakulam, 9(1):34-41
- Shenoy, A.V. and V.K. Pillei, Freezing characteristics of tropical fishes. 1. Indian oil sardines.
1971 Fish.Technol.Soc.Fish.Technol.,Ernakulam, 8(1):37-41
- Slevin, J.W., Frozen fish: characteristics and factors effecting quality during freezing and
1968 storage. In The freezing and preservation of foods, edited by D.K. Tressler, et al.,
Westport, Connecticut, AVI Publishing Co., pp. 179-96
- Sreenivasan, N., et al., Mysore J.Agric.Sci., 10:296-30
1976
- Stenaby, M.E., Properties of fish oils, etc. In Chemistry and biochemistry of marine food
1982 products, edited by R.E. Martin, et al., Westport, Connecticut, AVI Publishing Co.,
pp. 87-8
- Teuchiye, Y. and Y. Tetsukawa, Green meat of swordfish. Tobutu J.Agric.Res., 4(2):183 (World Fish.
1954 Abstr., 1955 May/June 33)

Table 1

References to frozen storage life of fish species

Order	Family	Species	Common name	Catching area	Reference
Clupeiformes	Clupeidae	<i>Sardinella longiceps</i>	Oil sardine	India	Mahen, Chaudhuri and Pillai, 1966; Pawar and Nagar, 1966; Bose, 1969; Kaimal, 1969; Shenoy and Pillai, 1971
Lamniiformes	Galeidae	<i>Galeus glaucus</i>	Shark	Not stated	Bruno, 1953
	Lauridae	<i>Larus nansu</i>	Shark	Not stated	Bruno, 1953
Myctophiformes	Harpodontidae	<i>Harpodon naluensis</i>	Bombay duck	India	Radhakrishnan, 1973
Parciformes	Carangidae	<i>Curupe melampygus</i>	Jack mackerel	India	Bose, 1969
	Cichlidae	<i>Tilapia mosambica</i>	Tilapia	India	Shenoy and Jones, 1972
	Istiophoridae	<i>Makaira indica</i>	White marlin	India	Bose, 1969
		<i>Makaira nigricans</i>	Blue marlin	India	Bose, 1969
	Kaewonidae	<i>Katsuwonus pelamis</i>	Skipjack	Pacific	Bito, 1964, 1965, 1969; Washimoto, 1963; Ohta et al., 1967
	Latjanidae	<i>Pristigaster flavipinnis</i>		Vanuatu	This paper
		<i>Pristigasteroides maitidensis</i>		Vanuatu	This paper
	Pracanthidae	<i>Priacanthus hamur</i>	Rigeye	Seychelles	King and Poulter, 1984
	Scombridae	<i>Rastrelliger brachysoma</i>	Chub mackerel	Brunei	Poulter, 1978
		<i>Rastrelliger kaudurua</i>	Indian mackerel	India	Bose, 1969; Sreenivasan et al., 1976; Jadhav and Mahat, 1970
		<i>Rastrelliger neglectus</i>	Chub mackerel	Thailand	Kay, Rattagool and Hardy, 1972
	Scombaromidae	<i>Scomberomorus commersoni</i>	Ser	India	Bose, 1969; Hirunmath and Sranivasan, 1979
		<i>Scomberomorus guttatus</i>	Spotted sear	India	Shenoy, 1976
Xiphiidae	Xiphiidae	<i>Xiphus glaucus</i>	Swordfish	Pacific	Asano and Taniya, 1953; Dyer, 1969

QUALITY CHANGES IN FISH CAUGHT OFF THE COAST OF PENINSULAR
MALAYSIA: FROZEN STORAGE OF CHUB MACKEREL (*Rastrelliger*
kanagurta), YELLOW-BANDED TREVALLY (*Selaroides leptolepis*)
AND THREADFIN BREEM (*Hemipterus tolu*)

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ABSTRACT

Proximate analysis, objective and organoleptic tests were performed on Malaysian chub mackerel, yellow-banded trevally and threadfin bream. Samples of gutted and ungutted fish were fast and slow frozen, and stored and glazed at -20°C for 16 to 24 weeks. At intervals various chemical indices of deterioration were determined and taste panel assessment performed. The data on lipid oxidation, protein denaturation and pH changes were related to textural, flavour, odour and general acceptability results.

1. INTRODUCTION

Fish is the primary source of animal protein in many countries of the world, feeding far more millions than either meat or milk (Borgstrom, 1962).

Being susceptible to spoilage, fish requires special techniques to maintain good quality. Freezing is one of the most effective methods for long-term storage for fish. Freezing at sea has become essential for maintaining quality of fish stored for long periods in many temperate countries.

The fishing industry is also faced with problems which arise from consumer demands, fluctuations in supply and seasonal factors. Freezing seems to be one method which can provide a solution to this problem by maintaining a balance between demand and supply.

At present the market for frozen fish is limited in Malaysia as fresh fish are readily available. However, and increasing demand for frozen fish can be anticipated due to urbanization which has resulted in an expanded market for other frozen foods. Furthermore, there is a potential export market for quality frozen fish and shellfish (Abdullah, Yu and Che Mood, 1982).

The literature contains a wide variety of methods that have been applied to measuring quality changes in frozen fish. However, it is evident that due to a number of factors (e.g., seasonal changes, size, rate of freezing, pH, and pre-freezing history) assessing the quality of frozen fish is very complex.

For evaluation of fisheries research and for standardized quality control of fishery products, an objective criterion is a necessity. Individual objective tests, however, can determine only a single aspect, e.g., flavour or texture, whereas a trained sensory panel can render judgement on several properties. Therefore, a combination of tests should be used to obtain information on texture, flavour and freshness.

Relationship between various tests have been proven statistically and several correlations published (Connell and Howgata, 1968; Connell, 1968; Moorjani *et al.*, 1962; Gutschmidt, 1963). Because of the complexity of the changes that take place during frozen storage of fish and the relationship between objective and sensory assessment, it will probably be necessary to carry out more than one test.

Most of the published data on frozen fish are on cold and temperate water species. Published studies on frozen tropical pelagic species include oil sardines (Shenoy and Pillsai, 1971), Bombay duck (Radhakrishnam, Solank and Ventakaraman, 1973), spotted saerfish (Shenoy, 1976) and chub mackerel (Bose, 1969; Keay, Ratagool and Hardy, 1972; Nair, Gopakumar and Nair, 1976).

Fatty fish like sardines, mackerel and seerfish undergo lipid changes during storage in ice and also during frozen storage (Bose, 1969). The shelf life of quick frozen oil sardines was limited by the fat content which showed seasonal variations (Bose, 1969). Ascorbic acid water glaze was found to be effective in retarding the oxidation of fat in other Indian fish (Sawant and Magar, 1961).

Keey, Retagool and Hardy (1972) working on chub mackerel (*Rastrelliger kanagurta*) from Thailand found a difference in rate of protein solubility at -14 and -30°C storage temperature; vacuum packaging and glazing have protective effects. They also found in the same experiment that thiobarbituric acid (TBA) values increase throughout storage at -14°C. Poulter (1978a) working on chub mackerel (*Rastrelliger brachyama*) from Brunei, found that the rate of freezing has no significant effect on product acceptability. Unlike temperate mackerel species, he found that little protein denaturation occurred in the samples stored at -10°C but concluded that deterioration of lipids was the most important factor affecting quality.

This project was undertaken to investigate the frozen storage stability of some Malaysian fish. In this work, the effect of the rate of freezing, pre-freezing (gutting) and post-freezing (glazing) treatments and time of storage were evaluated organoleptically as well as objectively. The findings of this project may allow more economic conditions to be adopted in commercial practice.

2. MATERIALS AND METHODS

2.1 Raw Materials

The fish used were caught off the coast of Peninsular Malaysia. Species used were chub mackerel (*Rastrelliger kanagurta* Cuvier), yellow-banded trevally (*Selaroides leptolepis* Cuvier) and threadfin bream (*Nemipterus tolu* Cuvier and Val.).

Chub mackerel was gutted and washed and placed in an insulated box. The other species were not gutted. Fish to ice ratio used was 1:1. The boxes were transported to the laboratory, on the same day and upon arrival (8 h later) the boxes were placed in a chilled room (1-0°C). The following morning (08:00 h), the fish were divided into sample lots of gutted, ungutted, glazed and unglazed. For glazed samples, the fish were sealed in plastic bags containing 1 litre of water. Half of the fish were quick frozen to -20°C in an air blast freezer and the other half in a still air freezer at -20°C. Samples of both quick and slow-frozen fish were sealed in plastic bags and stored at -20°C in a still air freezer. At regular intervals, the fish were removed from storage and thawed at room temperature (27°C). The fish were then filleted and suitable portions of the fillet were put aside for organoleptic analysis. After the dark muscle, skin and large pieces of connective tissue had been removed the remaining fish were finely chopped with a scalpel and scissors. This finely chopped white muscle was maintained at 0°C until analysis.

2.2 Proximate Analysis

Moisture: approximately 5-g portions of homogenized muscle were placed in a convection oven at 105°C for 12 h and weight loss recorded (Pearson, 1970).

The fat content was determined by extracting the residue obtained from the moisture determination with light petroleum ether in a Soxhlet extractor (Pearson, 1970).

Ash: three grams of muscle were placed in a silica dish and charred using a Bunsen burner. The charred sample was ashed in a muffle furnace at 5 000°C (Pearson, 1970).

Crude protein was determined on 1-g samples of fish muscle using the micro-kjeldahl method (Bredstreet, 1965) using a factor of 6.25 (Pearson, 1970).

Ten grams of muscle were homogenized in 100 ml water (neutralized with 0.005 M sodium iodacetate to pH 7). The pH was measured at room temperature using a digital pH meter (Philips model PW 9408), with a glass electrode.

Protein solubility was determined using 3 g of homogenized muscle in 5% NaCl (Cowie and Little, 1967). Two millilitres of the filtrate were used to determine protein using the semi-micro-kjeldahl method (Pearson, 1970). A factor of 6.25 was used. Values were calculated as soluble protein expressed as percentage of total protein.

Thiobarbituric Acid Values (TBA) of 20-g samples were determined by malonaldehyde extraction method of Vyncke (1973). Optical density was determined at 538 nm using a Spectronic 20 Bausch and Lomb Spectrophotometer. The values were expressed as mg/kg sample.

2.3 Sensory Evaluation

Six trained panelists were used to assess the acceptability of the raw and cooked fish. The raw fish was examined to assess the condition of the eyes, gills, flesh and skin (Howgate, 1978).

Thawed fish were filleted into portions weighing approximately 25 g and hermetically sealed in polyethylene bags and boiled for 20 min. The panelists used a scale of 1 to 5 for texture and 1 to 10 for flavour, odour and general acceptability scores; 10 (or 5 for texture) being good, 1 being rejected, 4 (or 2 for texture) being just acceptable.

3. RESULTS AND DISCUSSIONS

3.1 Chemical Composition of Muscle

The proximate composition of the flesh of the fish species (Table 1) is typical of that of many species of fish. The moisture content of the fish species ranged from 68.3 to 79.8% and lipid content from 0.8 to 9.7%. The lipid content was relatively low and the moisture content high, with the slight exception of trevally (*Selaroides leptocephalus*). Despite the fact the chub mackerel (*Rastrelliger kanagurta*) is termed a fatty fish, the lipid content was relatively low (2.12%). It is now well established that there is inverse linear relationship between lipid and water content in fatty fish and the relative proportions of these two components vary depending on the season of the year and place of nutrition (Love, 1970). Previous studies by Abdullah and Yu (1979) on chub mackerel revealed a variation in water and lipid content showing a similar inverse linear relationship.

The species studied here must be considered as lean species. Trevally (*Selaroides leptocephalus*), however, could be considered a fatty fish because of its relatively higher fat content (5%). Unlike the others which are pelagic species, threadfin bream (*Semiprimerus tolu*) is a demersal fish and their flesh lipid contents were less than 1%.

The protein content was between 15.7 to 19.6%. The sum of the percentage of water, protein, fat and ash was below 100. This may be due to the low factor used for converting nitrogen to protein, particularly in the species studied which may contain a high proportion of protein nitrogen. Van de Velde (1932) estimated that the conversion factor should be in the neighbourhood of 8. Praga (1956) fixed it at 7 for muscles. On the other hand Causeret (1950) considers 6.25 to be too high, the nitrogen of fish not being completely built into protein molecules. The chemical composition of the flesh of the different species studied were in good agreement with those found in the literature (Poulter, 1978a).

3.2 Protein Solubility, pH and Texture

Figure 1 shows the percent soluble protein nitrogen (% SPN) of the three species in 5% NaCl solution at -20°C. As usual with % SPN determinations, the scatter in the values is high (Connell, 1968). For chub mackerel, in relation to this scatter, there is no detectable difference in the % SPN levels in the fast and slow frozen samples for both glazed and unglazed, and more surprisingly, no large decrease with time of storage at -20°C. Poulter (1978a) reported similar observations on chub mackerel (*Rastrelliger brachyura*) caught off the coast of Brunei. Keay, Ratagool and Hardy (1972) reported (on chub mackerel from Thailand) that % SPN values fell to 60% of the initial value in samples stored at -14°C and 80% in samples at -30°C, during storage for 6 months.

The ungutted trevally samples showed a steeper drop in % SPN as compared to the gutted samples at the initial period of storage. However, at the end of 6 months of storage all samples showed a drop to between 38-63% of the initial value. Fast freezing of gutted fish showed slightly less protein denaturation than slow freezing. On the other hand, the protective effect of glazing was more prominent in reducing protein denaturation in the case of ungutted trevally.

In the case of threadfin bream, there was a decrease in % SPN for the first 3-6 weeks of storage and then an increase to a slightly higher level at 9-12 weeks after which the values dropped quite slowly to the initial value or just below it. For gutted fish, the unglazed samples seemed to show slightly higher values than glazed samples. In ungutted fish, the faster rate of freezing seemed to indicate higher solubility.

Trials in India (Bose, 1969) with sardines (*Sardinella longiceps*) indicated that the % SPN values after 6 months storage were 70 % of initial value in samples stored at -12.5 °C and 80 % in samples stored at -23 °C. Investigations on cold water fish (Love, 1966) indicated that the % SPN of fish muscle falls only very slowly during storage at low sub-zero temperature. For example, in cod (*Gadus morhua*) stored at -30°C it takes nearly ten years for the % SPN to fall to half its initial value (Love, 1966). On the other hand, at high sub-zero temperature the % SPN value falls much more rapidly, taking only two months at -8°C for the % SPN values to fall by 50 % in herring (*Clupea harengus*), a cold water pelagic fish (Poulter and Lawrie, 1977).

It may be possible that the deterioration of proteins in frozen tropical fish occur at a slower rate than those in temperate or cold water fish. In this respect it is now well known that the spoilage of tropical fish in ice occurs at a much slower rate than the cold water fish (Disney, Cola and Jones, 1974).

In cold water fish the texture of the muscle has been found to be dependent on the pH and % SPN (Cowie and Little, 1967). The changes in pH during frozen storage of the three species are shown in Table 2. The pH of chub mackerel decreased by approximately 0.1 of a pH unit, that of trevally by 0.2-0.5 pH unit and that of threadfin bream increased by 0.2-0.5 pH unit.

Poulter (1978a) reported that the pH of chub mackerel remained constant during frozen storage. In view of the little change in pH and % SPN for chub mackerel, it would be expected that the texture of the fish would have remained fairly constant. However, taste panel results (Table 3) did not support this view. The texture score for all samples decreased markedly with increase in time of storage. This may support the view that protein denaturation (indicated by % SPN) could be assumed to be arrested when the temperature falls below the cryohydratic point of NaCl (-21.4°C), though texture changes may develop at this temperature (Love, 1985). Therefore pH had little influence on the textural deterioration of chub mackerel under storage at -20°C. This is in contrast to the findings of Cowie and Little (1966), who found that pH is a major factor affecting the texture of frozen fish.

Similarly for trevally, the slightly greater decrease in pH (0.2-0.5 units) reflected the rate of protein denaturation (as shown by % SPN) in all samples. Texture scores (Table 4) of cooked samples showed a reduction over a period of 6 months. This conformed to the values of % SPN and pH changes which influence the textural score. However the product was texturally acceptable at the end of the storage period.

In contrast to the previous two species, the pH of threadfin bream increased during storage by 0.2-0.5 units. The best pH (in cod flesh) for long-term frozen storage was 6.7 or above (Kelly, 1969). High pH has been related to depletion of tissues (Love, 1969) and a softer product. A relatively higher pH and % protein insolubility indicated that more denaturation had taken place which resulted in a softer product (Table 4). After 21 weeks of storage, the texture score was between 2.7-3.0, a figure considered texturally acceptable but on the softer side.

3.3 TBA, Odour and Flavour

The deterioration of flavour in fish muscle during frozen storage is caused by a complex series of reaction and changes such as development of rancidity as well as degradation of nucleotides (Hiltz, et al., 1971). In the rancid fat the compound formed which reacts with TBA is malonaldehyde (Sinnhuber, Yu and Yu, 1958). Correlation between TBA test with sensory judgement has been reported to be favourable (Ryan and Stansby, 1959; Anderson and Dannielson, 1961).

Figure 2 shows the malonaldehyde values per kilo of chub mackerel, trevally and threadfin bream stored at -20°C. The results indicated that for chub mackerel, the values increased up to 16 weeks of storage. However, for trevally and threadfin bream, the values increased initially but gradually decreased after 9-12 weeks. In all cases the unglazed samples seemed to have higher values than glazed samples.

Despite the fact that the lipid content of threadfin bream was low (0.8%, Table 1), the trend in the production of malonaldehyde was quite similar to trevally which had a much higher lipid content of 9.7% (Table 1). The malonaldehyde production at the end of 24-week storage for threadfin bream was 2.5 mg/kg and for trevally less than 5.0 mg/kg. In contrast to chub mackerel, which had a lipid content of 2.1% (Table 1), the malonaldehyde production was between 14-17 mg/kg sample after only 16 weeks storage.

In the case of both trevally and threadfin bream, the lower recorded malonaldehyde production may indicate that the fatty acids have oxidized. But instead of accumulating into aldehyde (which gives rise to rancid odours and flavours), they are "removed" by combining into insoluble lipid-protein polymer (Gould and Peters, 1971). This may affect the protein solubility and consequently the texture of the fish.

Tables 3 and 5 show odour and flavour scores respectively of cooked threadfin bream over the period of storage at -20°C. The results indicated that all samples were just acceptable after 21 weeks storage. The results are quite comparable to those found in trevally (Tables 3 and 5) over the same period of storage, despite the originally higher lipid content (9.7%) of the latter species. However, the results of chub mackerel having intermediate lipid content of 2.1% showed much lower odour and flavour scores (Tables 3 and 5) over a shorter storage period of 16 weeks.

Lipid oxidation precedes protein insolubilization (Dyer and Frazer, 1959) and neutral fats help to protect the proteins against insolubilization due to lipid oxidation (Hanson and Olley, 1965). Protein stability was greatest for fatty fish and least in lean fish (Anderson and Steinburg, 1964). The proportion of polyunsaturated fats in the phospholipids, was considered to be the prime source of oxidative rancidity particularly in the dark flesh, which has a high proportion of haem compounds.

Notched threadfin bream (*Hemipterus tolu*, Cuvier and Val.) may be considered a lean fish (0.8% fat). The protein denaturation in this fish seemed to occur rapidly and substantially, as early as three weeks after frozen storage. One possible reason for the rapid denaturation could be that less neutral lipid is "available" (fat content only 0.8%) to counteract the influence of fatty acids on protein insolubilization (Hanson and Olley, 1965). The texture of the product was also found to be on the softer side at the end of the storage period. This was supported by a relatively

higher recorded pH values, which resulted in soft products. The increase in protein insolubilization by fatty acid interactions is also influenced by high pH (Anderson, Steinberg and King, 1965). Therefore in threadfin bream, the low fat content and a relatively high pH of the product added up to decreased protein solubility of the muscle which in turn influenced the softer texture of the muscle.

In comparison to chub mackerel (*Rastrelliger kanagurta*, Cuvier) which has a slightly higher fat content (2.1%), the protein denaturation occurred much slowly and incompletely. This is evident in the nature of the protein solubility curve (Figure 1). It is possible that the amount of neutral lipids "available" was sufficient to counteract the effect of fatty acid oxidation on protein insolubilization (Hanson and Olley, 1965). Supplemented by a lower recorded pH values in chub mackerel, the product of the frozen samples gave less soft texture.

In the case of yellow-banded trevally (*Selaroides leptolepis*, Cuvier), which may be considered a fatty fish (9.7% fat), the % protein insolubility took a large drop in the beginning and remained relatively low throughout the storage period. The occurrence is about similar to the case in threadfin bream with low fat content. This phenomena may be explained (Hanson and Olley, 1965) by the fact that the high fat content in trevally may be present in the "globular" or "depo" but not "available" to mitigate the effect of insolubilization of protein due to fatty acid oxidation. This phenomena could also be supported by a similar high recorded pH value resulting in a less firm product, though acceptable at the end of the storage period.

In terms of rancidity and its effect on the flavour and odour scores, comparing the three species chub mackerel, yellow-banded trevally and notched threadfin bream, despite the fact the latter two species contain high and low fat contents respectively, the recorded malonaldehyde production for both was relatively low. This resulted in higher scores for both odour and flavour. In the case of the former species, chub mackerel, which contains a medium amount of fat, recorded higher values of malonaldehyde production, which resulted in lower scores for both flavour and odour. This comparison indicated that rancidity and hence its effect on flavour and odour does not reflect the total fat content but perhaps the nature of the fat itself. Furthermore part of the products of fat oxidation may form lipid-protein polymers making it less available to be detected and hence reduce its influence on flavour and odour. However in the former case (chub mackerel), the influence is more on the % SPN and hence textural changes as compared to its influence on flavour and odour.

In terms of general acceptability (Table 6) threadfin bream and trevally gave higher scores over that of chub mackerel at the same storage period (16 weeks). Lengthening the storage period for another 5 weeks in the case of threadfin bream and 8 weeks in the case of yellow-banded trevally would make the product acceptable similarly to chub mackerel at only 16 weeks storage period. The interactions between lipid and protein could have made these differences possible despite the variations in fat contents of the species under study.

4. REFERENCES

- Abdullah, M.I. and S.Y. Yu, The effect of freezing and frozen storage on the quality of chub mackerel (*Rastrelliger kanagurta*). In Proceedings of the Symposium on Protein rich food in ASEAN, edited by M. Zahara et al., Kuala Lumpur, Malaysian Institute of Technology, p. 146
- Abdullah, M.I., S.Y. Yu and M.R. Che Hood, Some quality changes in frozen chub mackerel (*Rastrelliger kanagurta*). In Proceedings of the International Symposium on food technology in developing countries, edited by S.K. Berry, et al. Serdang, Selangor, Department of Food Science and Technology, Universiti Pertanian Malaysia
- Anderson, K. and C.E. Danielson, Storage changes in frozen fish. A comparison of objective and subjective test. *Food Technol.*, 15:55-7.
- Anderson, M.L. and M.A. Steinberg, The effect of lipid content on protein-sodium linoleate interaction in fish muscle homogenate. *J. Food Sci.*, 29:327
- Anderson, M.L., M.A. Steinberg and F.J. King, Some physical effects of freezing of fish muscle and their relation to protein fatty-acid interaction. In Technology of fish utilisation, edited by R. Kreuzer. London, Fishing News (Books) Ltd., for FAO, pp. 105.
- Borgstrom, G., Fish is world nutrition. In Fish as food, edited by G. Borgstrom. New York, Academic Press, pp. 267-360
- Bose, A.N., Freezing of tropical fish. In Freezing and irradiation of fish, edited by R. Kreuzer. London, Fishing News (Books) Ltd., for FAO, pp. 179-88
- Bradstreet, R.B., The Kjeldahl methods for organic nitrogen. London, Academic Press 1965

- Cauereet, J. Les éléments minéraux des poissons. Bull.Soc.Sci.Hyg.Aliment, 38:1-39
1950
- Connell, J.J., The effect of freezing and frozen storage on the proteins of fish muscle. In Recent
1968 advances in food science, edited by J. Hawthorn and E.J. Rolfe. Oxford, Pergamon Press,
vol. 4:333
- Connell, J.J. and P.F. Howgate, Sensory and objective measurement of the quality of frozen cod of
1968 different initial freshness. J.Sci.Food Agric., 19:342
- Cowie, W.F. and W.T. Little, The relationship between the toughness of cod stored at -29°C and its
1966 muscle protein solubility and muscle pH. J.Food Technol., 1:335
- _____, The relationship between the toughness of cod stored at -7 and -14°C, its muscle
1967 solubility and muscle pH. J.Food Technol., 2:217
- Disney, J.C., R.C. Cole and N.R. Jones, Considerations in the use of tropical fish species. In
1974 Fishery products, edited by R. Kreuzer. London, Fishing News (Books) Ltd., for FAO,
pp. 329-37
- Dyer, W.J. and D.I. Frazer, Protein in fish muscle. 13. Lipid hydrolysis. J.Fish.Res.Board Can.,
1959 16(1):43-52
- Praga, P.C., Variaciones estatales en la composición química de almejas (*Mytilus edulis*).
1956 Invest.Pesqu.,Barc., 4:109-25
- Gould, E. and J.A. Peters, On testing the freshness of frozen fish. London, Fishing News (Books)
1971 Ltd., p. 35
- Gutschmidt, J., Organoleptic and physical methods to determine the texture of fish. Proc.Int.Cong.
1963 Refinger., 11
- Hanson, S.W.P. and J. Olley, Observations on the relationship between lipid and protein deteriora-
1965 tion. In The technology of fish utilisation, edited by R. Kreuzer. London, Fishing
News (Books) Ltd., for FAO, pp. 111-5
- Hilts, D.F., et al., Variation of biochemical quality indices by biological and technological
1971 factors. In Fish inspection and quality control, edited by R. Kreuzer. London,
Fishing News (Books) Ltd., for FAO, pp. 191-5
- Howgate, P.F., Measurement of deterioration of iced and frozen fish. Course Note (TD 564) Torry
1978 Res.Stn., Aberdeen
- Keay, J.N., P. Ratagool and P. Hardy, Chub mackerel of Thailand (*Rastrelliger neglectus* van kamp):
1972 A short study of its chemical composition, cold storage and canning properties.
J.Sci.Food Agric., 23:1359
- Kelly, K.O., Factors affecting the quality of frozen fish. In Freezing and irradiation of fish,
1969 edited by R. Kreuzer. London, Fishing News (Books) Ltd. for FAO, pp. 339
- Love, R.M., Studies on protein denaturation in frozen fish. 3. The mechanism and rate of denatura-
1958 tion at low temperature. J.Sci.Food Agric., 9:609
- _____, The use of tasters for investigating cold storage deterioration in frozen fish.
1966 J.Food Technol., 1:141
- _____, Condition of fish and its influence on the quality of frozen products. In Freezing
1969 and irradiation of fish, edited by R. Kreuzer. London, Fishing News (Books) Ltd., for
FAO, pp. 40
- _____, The chemical biology of fishes. London, Academic Press, 262 p.
1970
- Noorjani, M.N., et al., Post-rigor changes in nitrogen distribution and texture of fish during
1962 storage in crushed ice. Food Technol.,Champaign, 16:80
- Nair, P.G.V., K. Gopakumar and N.R. Nair, Lipid hydrolysis in mackerel (*Rastrelliger kanagurta*)
1976 during frozen storage. Fish Technol.Soc.Fish.Technol.,Ernakulam, 13:111
- Peareon, D., The chemical analysis of foods. London, J. and A. Churchill, 6th ed.
1970

- Poulter, R.G., Quality changes in fish from South China Sea: frozen storage of chub mackerel.
1978 Proc.IPFC, 18(3):330-9
- Poulter, R.G. and R.A. Lawrie, Studies on fish muscle protein. Nutritional consequences of the
1977 changes occurring during frozen storage. J.Sci.Food Agric., 28:701
- Poulter, R.G., L. Nicolaides and D.A. Hector, Quality changes in fish from the South China Sea:
1978 iced storage of chub mackerel, grouper and fusilier. Proc.IPFC, 18(3):169-85
- Radrakrishnam, A.G., K.K. Solank and R. Ventakaraman, Preliminary studies on freezing characteris-
1973 tics of Bombay duck (*Harpodon netherese*). Fish.Technol.Soc.Fish.Technol., Ernakulam,
10:124
- Ryan, B.A. and M.E. Stansby, Measurement of rancidity in fish products by 2-thiobarbituric acid
1959 method. Commer.Fish.Rev., 21(1):21
- Sawant, P.L. and N.G. Magar, Frozen fish. 1. Denaturation of proteins. J.Food Sci., 26:253
1961
- Shenoy, A.V., Freezing characteristics of tropical fishes. 3. Spotted seer (*Scomberomorus guttatus*).
1976 Fish.Technol.Soc.Fish.Technol., Ernakulam, 13:105
- Shenoy, A.V. and V.K. Pillai, Freezing characteristics of tropical fishes. 1. Indian oil sardines.
1971 Fish.Technol.Soc.Fish.Technol., Ernakulam, 8:105
- Sinnhuber, R.O., T.C. Yu and C.T. Yu, Characterisation of red pigment formed in the 2-thiobarbituric
1958 acid determination of oxidative rancidity. Food Res., 23:627-33
- Van de Velde, A.J.J., Investigation on the chemical composition of fish. 3. Composition of
1932 *Pleuronectus* and *Scomber*. Natuurw.Tijdschr.Ned.-Indie, 14:178
- Vyncke, W., Evaluation of the direct thiobarbituric acid extraction method for determining oxidative
1973 rancidity in mackerel (*Scomber scombrus*, L.). Paper presented at the meeting of the
Joint FAO/WHO Expert Committee on Fish and Shellfish Hygiene, Geneva

Table 1

Chemical composition of fish used

Species	Moisture %	Fat %	Protein %	Ash %
Chub Mackerel (<u>Bastrelliger</u> <u>Kanagurta</u> , cur.)	73.40(72.8-76.3)	2.08(1.5-2.7)	22.03(20.8-24.6)	2.50(2.03-2.95)
Yellow banded Trevally (<u>Scleroides leptolepis</u> , cur.)	68.30(67.8-68.7)	9.70(8.05-12.9)	18.70(13.03-20.7)	1.80(1.7-1.9)
Notched threadfin bream (<u>Nemipterus tolu</u> , cur. & Val.)	77.50(76.85- 77.90)	0.80(0.68-0.83)	17.70(16.23-18.95)	1.90(1.73-2.04)

Table 2
pH changes in chub mackerel, trevally and threadfin bream during
frozen storage at -20°C

TIME OF STORAGE (WEEKS)	CHUB MACKEREL				YELLOW-BANDED TREVALLY								THREADFIN BREEM							
	Quitted				Quitted				Unquitted				Quitted				Unquitted			
	FG	SU	SG	PU	FG	SU	SG	PU	FG	SU	SG	PU	FG	SU	SG	PU	FG	SU	SG	PU
0	6.25	6.25	6.25	6.25	6.83	6.83	6.83	6.83	6.83	6.83	6.83	6.83	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55
2	6.30	6.30	6.31	6.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	6.62	6.75	6.88	6.87	6.73	6.70	6.67	6.79	6.93	6.94	6.66	6.99	6.80	6.85	6.88	6.88
4	6.30	6.30	6.36	6.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	6.25	6.25	6.27	6.23	6.33	6.88	6.95	6.93	6.53	6.75	6.78	6.77	6.89	6.95	7.05	6.93	6.89	6.92	6.77	6.77
8	6.24	6.28	6.27	6.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	6.35	6.85	6.80	6.85	6.45	6.60	6.70	6.82	6.90	6.85	6.99	6.76	6.81	6.92	6.79	6.79
10	6.22	6.28	6.26	6.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	6.15	6.15	6.18	6.19	6.25	6.45	6.35	6.40	6.40	6.45	6.30	6.81	6.60	6.84	6.88	6.72	6.95	6.91	6.86	6.86
14	6.14	6.12	6.19	6.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	6.03	6.10	6.10	6.16	6.20	6.75	6.30	6.83	6.47	6.73	6.87	6.87	6.74	6.77	6.89	6.89
16	6.12	6.18	6.13	6.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	6.10	6.20	6.40	6.25	6.28	6.35	6.38	6.99	6.77	6.77	6.87	6.94	6.72	6.87	6.79	6.79
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	6.72	6.51	6.85	6.50	6.35	6.38	6.35	6.77	6.76	6.77	6.77	6.87	6.87	6.83	6.71	6.71
24	-	-	-	-	6.35	6.60	6.62	6.35	6.78	6.38	6.35	6.35	-	-	-	-	-	-	-	-

Note: FG - Fast glazed

SG - Slow glazed

PU - Fast unglazed

SU - Slow unglazed

Table 3

The effect of storage at -20°C on the odour scores in samples of chub mackerel, yellow-banded trevally and threadfin bream

TIME OF STORAGE (WEEKS)	CHUB MACKEREL						YELLOW-BANDED TREVALLY						THREADFIN BREAM					
	Gutted			Gutted			Gutted			Gutted			Gutted			Gutted		
	FG	SU	SG	FG	SU	SG	FG	SU	SG	FG	SU	SG	FG	SU	SG	FG	SU	SG
0	9.7	9.7	9.7	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	8.2	8.2	8.2	8.2	8.2	8.2
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	10.0	9.5	10.0	10.0	9.5	9.0	10.0	10.0	9.0	9.0	8.5	8.5	8.2	8.3	8.2
4	8.2	9.3	7.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	9.0	9.0	9.0	9.0	8.0	9.0	7.7	8.0	7.5	8.0	7.8	7.8	7.5	7.5	7.5
8	6.3	6.1	6.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	6.8	7.0	6.8	7.0	8.0	7.0	8.0	8.2	8.3	9.0	8.8	8.2	8.0	8.8	8.5
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	4.3	5.0	4.3	7.6	7.6	7.2	6.8	7.6	7.6	6.8	6.8	7.7	7.8	8.0	7.5	8.0	7.3	7.2
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	7.5	6.8	7.5	7.0	6.0	7.5	7.5	7.5	7.0	7.0	7.0	6.8	7.0	7.0	6.8
16	2.4	2.8	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	7.0	7.0	6.8	6.8	6.5	7.0	6.8	7.0	6.7	6.7	6.7	6.5	6.3	6.3	6.7
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	6.0	6.0	6.0	5.2	6.0	6.0	6.0	6.0	5.7	5.7	6.0	5.5	5.8	5.8	5.5
24	-	-	-	6.0	5.4	5.6	5.4	5.6	6.0	5.6	5.2	-	-	-	-	-	-	-

Notes: FG - Fast glazed

SG - Slow glazed

FU - Fast unglazed

SU - Slow unglazed

Table 4

The effect of storage at -20°C on the texture score in samples of chub mackerel, trevally and threadfin bream

TIME OF STORAGE (#EXS)	CHUB MACKEREL						YELLOW-BARRA/ TREVALLY						THREADFIN BREAM					
	Outgated			Outgated			Outgated			Outgated			Outgated			Outgated		
	FG	FU	SG	SU	FG	SG	FU	SG	SU	FG	FU	SG	FU	SG	SU	FG	FU	SG
0	4.8	4.8	4.8	4.8	4.0	5.0	4.2	4.0	4.2	5.0	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	3.5	4.0	4.0	3.5	3.7	4.2	4.0	4.0	4.2	4.0	4.0	4.0	4.0	4.2
4	4.0	4.2	3.8	4.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	3.5	3.2	4.0	3.2	4.0	3.2	4.0	4.0	3.5	3.8	3.8	3.5	3.5	3.5
8	3.8	3.0	3.8	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	3.2	3.2	3.0	3.0	3.0	3.0	3.0	3.0	4.2	4.2	3.8	4.0	3.7	4.2
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	3.6	2.8	3.0	3.2	3.2	3.2	3.0	3.2	3.2	4.0	3.2	3.2	3.8	3.8	4.2	4.0	3.8	4.2
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	3.0	3.0	3.0	3.0	3.0	3.4	4.0	3.4	3.0	3.0	3.0	3.0	3.0	3.0
16	3.2	2.4	2.8	2.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	2.2	2.2	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.2	2.8	3.0
24	-	-	-	-	2.2	2.0	2.0	2.2	2.2	2.0	2.0	2.0	2.0	2.8	3.0	2.8	2.7	2.8

Note: FG - Fast glazed SG - Slow glazed
FU - Fast unglazed SU - Slow unglazed

Table 5

The effect of storage at -20°C on the flavour scores in samples of chub mackerel, yellow-banded trevally and threadfin bream

TIME OF STORAGE (#2255)	CHUB MACKEREL						YELLOW-BANDED TREVALLY						THREADFIN BREAM					
	Gutted						Gutted						Gutted					
	FG	SU	SG	SU	FG	FG	FG	SU	SU	FG	FG	SU	FG	SU	FG	FG	SU	SU
0	9.1	9.1	9.1	9.1	9.0	9.0	9.0	9.0	9.0	9.0	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	8.2	8.2	8.2	8.5	9.0	8.5	9.0	9.0	8.3	8.0	8.0	8.0	8.0	8.0
4	6.6	7.4	7.0	7.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	8.4	7.5	9.0	8.4	9.0	7.5	7.8	8.0	7.8	7.8	7.8	7.8	8.0	8.0
8	5.4	6.1	5.7	5.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	7.5	7.5	8.0	8.0	8.0	8.0	7.5	8.0	8.3	7.5	7.5	7.5	7.5	7.5
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	4.6	3.5	5.5	5.3	6.8	7.0	7.5	7.3	7.5	7.5	6.7	7.0	7.3	7.3	7.3	5.8	6.8	7.3
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	7.5	7.6	7.0	7.2	7.0	8.0	6.6	6.6	6.4	6.6	7.0	6.2	6.8	6.6
16	4.1	4.3	4.0	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	6.8	7.0	6.8	6.4	7.0	7.0	6.5	6.5	6.7	6.7	6.3	6.7	6.5	6.5
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	6.0	5.2	5.2	6.0	6.0	5.0	5.0	5.2	5.2	5.3	5.0	5.7	5.0	5.0
24	-	-	-	-	5.4	5.0	4.8	4.8	5.2	5.0	-	-	-	-	-	-	-	-

Note: FG - Fast glazed

SG - Slow glazed

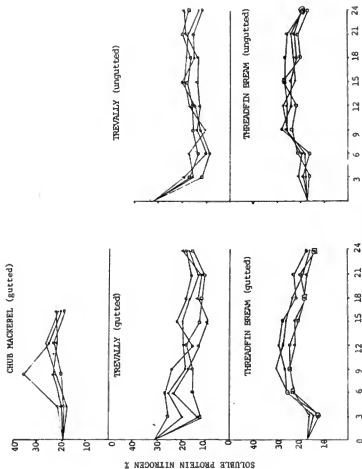
FU - Fast unglazed

SU - Slow unglazed

Table 6
The effect of storage at -20°C on the general acceptability scores in samples
of Chub mackerel, yellow-banded trevally and threadfin bream

TIME OF STORAGE (WEEKS)	CHUB MACKEREL						YELLOW-BANDED TREVALLY						THREADFIN BREEM					
	Outted			Outted			Ungritted			Gutted			Gutted			Ungritted		
	FC	FU	SG	SU	PG	FG	PU	SG	SU	PG	FU	SG	PU	FG	SU	PG	FU	SU
0	9.0	9.0	9.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.2	7.0	8.2	8.0	8.2
4	8.0	8.0	8.4	8.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	8.2	7.7	9.0	8.3	8.2	8.3	7.3	7.3	7.5	-	7.3	7.8	7.5	7.5
8	8.0	7.2	6.4	6.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	8.0	7.8	8.2	7.8	7.5	7.8	7.8	8.2	8.2	-	8.3	7.8	8.3	8.0
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	6.0	5.6	4.8	5.2	7.2	7.2	7.3	8.0	7.5	7.6	7.2	7.6	7.0	6.8	7.5	7.2	7.0	6.7
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	7.4	8.0	8.0	7.7	7.2	6.8	7.0	6.6	7.0	6.6	6.2	6.6	6.8	6.6
16	4.4	4.4	3.6	3.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	7.3	6.8	7.0	7.0	7.3	6.8	6.8	6.8	7.0	6.8	7.0	6.7	5.5	6.7
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	6.4	7.2	7.2	6.4	6.6	6.4	6.6	6.6	5.5	5.3	5.7	5.5	5.7	5.7
22	-	-	-	-	5.2	5.2	5.4	4.8	5.0	5.0	4.8	-	-	-	-	-	-	-

Note: FC - Fast glazed SG - Slow glazed
FU - Fast unglazed SU - Slow unglazed



TIME OF STORAGE (WEEKS)

Figure 1 The effect of storage at -20°C on the amount of Protein Soluble in 5% NaCl of frozen samples of chub mackerel, yellow-banded trevally and threadfin bream (Fast glazed (Δ - Δ), fast unglazed (○ - ○), Slow glazed (● - ●), Slow unglazed (- -)).

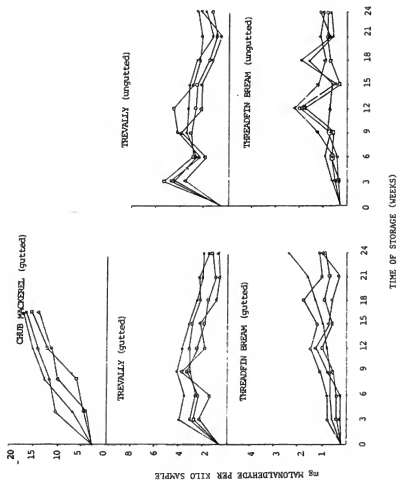


Figure 2 The effect of storage at -20°C on the TBA values of frozen chub mackerel, yellow-banded trevally and threadfin bream. (Fast glazed ($\Delta - \Delta$), fast unglazed (o - o), Slow glazed ($\square - \square$), Slow unglazed (. - .))

QUALITY OF FROZEN CHUB MACKEREL (*Rastrelliger kanagurta* Cuvier)
AFTER CANNING

by

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ABSTRACT

Fresh and frozen chub mackerel were canned to determine the quality changes during storage. Soluble protein nitrogen in 5% NaCl was not a good indicator of protein denaturation. Frozen samples have higher initial thiobarbituric acid values (TBA) before canning and the value increases with time of storage for both fresh and frozen samples. The difference in acceptability of canned frozen and fresh chub mackerel were only detectible after 1 month storage. Fish which has been frozen 2-3 months before canning were still acceptable after 3 months storage in cans, and the acceptability decreased with increase in time of frozen storage before canning.

1. INTRODUCTION

Whenever fish must be kept for more than a few hours before canning, chilling is the method of choice. Whenever the time lapse between catching and canning exceeds several days, bulk freezing is advisable for keeping raw material in good condition. Good initial quality, quick freezing, good glazing and low temperature are the principal factors governing the quality of the canned product (Van dan Broeck, 1965).

Norwegian sprats must be stored at -30 to 35°C if they are to be canned after 8-10 weeks of frozen storage with a quality comparable to that of newly caught canned sprats (Van dan Broeck, 1965). MacCallum et al. (1956) found that Yugoslavian sardines were still suitable for canning after 3 months of storage at -20°C.

Although it has been reported that the quality of canned product made from frozen fish is not different from the unfrozen material e.g., mackerel (Herdy, 1953), conclusions that differences do exist seem to predominate.

Occasionally the changes are for the better, e.g., when through protein denaturation the fish flesh acquires a firmer texture, as seems to be the experience of some herring canners (Van dan Broeck, 1965). Texture, instead of being improved by freezing, can also be impaired since toughness increases with longer frozen storage. A brine dip before freezing or even after thawing may reduce this kind of deterioration (Van dan Broeck, 1965).

A limit to frozen storage of salmon to be used for canning was put at 50 days by Tanikawa, Motohiro and Sheji (1956). A typical "freezer" odour, noted upon opening cans of salmon preserved by freezing, was found to be due to fat oxidation. This appeared after about 50 days of normal frozen storage. This period can be increased to about 80 days by treatment with butylated hydroxy-anisole (BHA) as an anti-oxidant (Tanikawa and Motohiro, 1959).

In the present study, the quality of chub mackerel which was frozen prior to canning was evaluated. Attempts were made to determine the quality changes due to freezing and frozen storage at -20°C before canning and their effect on the acceptability of the product after canning.

2. MATERIALS AND METHODS

2.1 Sampling

Chub mackerel (*Rastrelliger kanagurta* Cuvier) caught from South China Sea off the East Coast of Peninsular Malaysia were blast frozen whole and ungutted. At intervals of one month, the frozen fish were taken out from storage and canned in tomato sauce. At the same time canning was also done on freshly caught chub mackerel for comparative purposes.

2.2 Quality Assessment

Quality assessment was performed on the canned product after storage at ambient temperature. Objective quality tests conducted were: thiobarbituric acid (TBA) values (Vynnycky, 1973; Abdullah and Yu, 1983), percent soluble protein nitrogen (percentage SPN) in 5% NaCl (Connell, 1968; Cowie and Little, 1967; Pearson, 1970; Abdullah and Yu, 1983) and pH changes (Abdullah and Yu, 1983). Organoleptic tests were determined based on general acceptability (Howgate, 1978; Abdullah and Yu, 1983).

2.3 Canning in Tomato Sauce

The fish were beheaded, eviscerated and soaked in 10% brine for 30 min, followed by soaking in 0.06% acetic acid for 10 min. Three to four fishes (depending on the size of fish) were packed per can. The cans were exhausted for 10 min, drained, filled with hot tomato sauce (80°C), sealed and immediately retorted at 121°C (15 psi) for 68 min. After retorting cans were cooled, dried and stored at ambient temperature until analyzed.

3. RESULTS AND DISCUSSION

3.1 Chemical Changes

3.1.1 Changes in percentage SPN

Table 1 shows percentage SPN in 5% NaCl of canned chub mackerel (fresh and frozen) during storage. It is noted that no definite trend could be detected in the values of percentage SPN for the canned products. This could indicate that protein denaturation had already occurred during the canning process itself and little further change occurred during storage in cans. Comparing the percentage SPN across the frozen storage period of fish for different storage times after canning did not indicate any definite trend. Percent SPN was a poor indicator of quality in canned frozen chub mackerel due to the occurrence of protein denaturation during freezing and frozen storage.

3.1.2 Changes in TBA values

Table 2 shows the changes in TBA values of canned chub mackerel (fresh and frozen) during storage. The results indicated that in all samples, the TBA values increased with time of storage in cans, with the exception of canned fresh fish where the values remained almost constant.

Canned products containing fish which had been frozen stored for a longer period had higher TBA values. Initially, TBA values were low. At the end of 4 months storage in cans, the TBA values of fresh fish were found to be the lowest and the value of frozen fish increased with increase in the time of storage before canning. This shows that fat oxidation had taken place during frozen storage before canning and this may affect the odour and general acceptability of the canned product (Tadokawa and Motohiro, 1959).

3.1.3 Changes in pH

Table 3 shows the pH changes of canned chub mackerel (fresh and frozen) in tomato sauce during storage. The results showed that generally the pH decreased slightly with storage time. However the difference between fresh and frozen fish was slight. This may indicate that a slight change in pH has little effect on the overall texture of the product, even though frozen storage has been known to increase toughness due to a decrease in pH (Lova, 1962).

3.2 Changes in Sensory Attributes

3.2.1 General acceptability

Table 4 shows the general acceptability score of canned chub mackerel (fresh and frozen) after storage at ambient temperature. The results indicated that for canned fresh fish, the samples were still acceptable up to 4 months of storage. However, the score was found to be decreasing with time of storage.

For canned frozen fish, the scores given by the panelists were comparatively lower than canned fresh fish. The difference between the fresh and frozen samples was detected by the panelists from the first month of storage. The panelists gave low scores (less than 6.0) for frozen samples and scores tended to decrease further as frozen storage of fish before canning was extended. Fish frozen for 1-2 months before canning was acceptable for up to 3 months of storage in cans. For fish which were stored longer than 3-4 months, the scores indicated some degree of unacceptability even at zero month of storage in cans, and with increase in storage time the scores indicated almost complete unacceptability.

Generally it was noted that fresh fish gave better canned product as compared to frozen fish. However, fish which had been frozen 1-2 months before canning yielded acceptable products only up to 3 months of storage in cans.

Table 1

The effect of storage at ambient temperature on (percentage) soluble protein nitrogen (SPN) in 5% NaCl in canned samples of initially frozen chub mackerel

Time of storage (months)	% Soluble Protein Nitrogen in 5% NaCl				
	Fresh fish	Frozen fish			
		1 month	2 months	3 months	4 months
0	21.9	21.7	19.37	19.61	19.10
1	21.7	16.35	16.34	25.00	19.80
2	24.2	13.05	18.60	25.30	12.70
3	22.9	21.8	24.39	20.90	12.90
4	18.7	21.2	14.37	20.5	12.4

Table 2

The effect of storage at ambient temperature on TBA values (mg malonaldehyde/kg sample) in canned samples of initially frozen chub mackerel

Time of storage (months)	TBA values				
	Fresh fish	Frozen fish			
		1 month	2 months	3 months	4 months
0	2.3	2.3	2.7	3.9	2.7
1	2.7	1.9	3.6	3.7	3.4
2	2.3	3.9	2.3	4.1	4.6
3	2.3	1.6	4.3	4.2	4.8
4	2.3	4.2	3.7	4.7	4.9

Table 3

The effect of storage at ambient temperature on pH changes in canned samples of initially frozen chub mackerel

Time of storage (months)	pH changes				
	Fresh fish	Frozen fish			
		1 month	2 months	3 months	4 months
0	6.06	6.02	6.05	5.97	5.85
1	6.09	6.01	5.98	6.05	6.13
2	6.02	5.86	5.97	6.02	5.89
3	5.98	5.89	6.13	6.96	5.26
4	5.68	6.02	6.02	5.36	5.30

Table 4

The effect of storage at ambient temperature on general acceptability scores in canned samples of initially frozen chub mackerel

Time of storage (months)	General Acceptability Scores				
	Fresh fish	Frozen fish			
		1 month	2 months	3 months	4 months
0	6.1	5.9	5.4	4.5	4.0
1	5.5	5.5	4.3	4.3	3.7
2	6.7	5.8	4.3	4.2	3.3
3	6.7	5.2	5.0	3.0	1.0
4	5.2	4.8	2.7	1.3	1.0

4. CONCLUSION

Generally it is evident that differences in quality existed between canning of fresh chub mackerel and fish which were frozen initially. The difference was most apparent in the TBA values which affected odour and flavour of the final product. This resulted in the differences in the general acceptability score between the two samples of fresh and frozen chub mackerel. Increase in the time of frozen storage would further increase deterioration of initial quality before canning and resulted in a less acceptable canned product.

Previous work on salmon (Tanikawa, Motohiro and Shaji, 1956), which was used for canning, reported a typical "freezer" odour upon opening cans, due to fat oxidation. Tuna (Van dan Broeck, 1965) held in cold storage for 14 months showed discoloration on canning, the pink colour became brown due to pigmentation.

Protein denaturation during frozen storage also results in the formation of curd, a protein coagulate often found on top of canned fish. This is due to the impairment of the water binding properties of muscle protein by freezing denaturation. The increase in curd is thought to be related to the formation of soluble proteins during frozen storage (Schmidt and Idler, 1958; Stanaby and Dawson, 1957).

Though the advantage of preserving fish by freezing and frozen storage prior to canning operation is obvious, especially in tropical conditions, it is considered necessary to minimise the storage period. For frozen fish, it is necessary to minimise protein denaturation and fat rancidity by short storage times and low storage temperature. The two factors would produce undesirable canned product.

5. REFERENCES

- Abdullah, M.I. and S.Y. Yu, Some quality changes in frozen chub mackerel (*Rastrelliger kanagurta*).
1983 In Proceedings of the International Symposium on food technology in developing countries edited by S.K. Berry *et al.*, Malaysia, Department of Food Science and Technology, Universiti Pertanian Malaysia
- Connell, J.J., The effect of freezing and storage on the proteins of fish muscle. In Recent advances
1968 in food science, edited by J. Hawthorne and E. Rolfe. Oxford, Pergamon Press, Vol.4:333-58
- Cowie, W.F. and W.T. Little, The relationship between toughness of cod stored at -7 and -14°C, its
1967 muscle protein solubility and muscle pH. J.Food Technol., 2:217
- Hardy, E., Fish for the cannery. 16. The mackerel. Canning Indus., 23:30-1
1953
- Howgate, P.F., Measurement of deterioration of iced and frozen fish. Courses Nota Terry Res.Stn.,
1978 Aberdeen, (TD 564)

- Lova, R.M., Protein denaturation in frozen fish. 6. Cold storage studies on cod using the cell fragility method. J.Sci.Food Agric., 13:269-78
- MacCellum, W.A., et al., Quality of sardines (*Clupea pilchardus*) held unfrozen and frozen prior to canning. Food Technol., 10:432-8
- Pearson, D., The chemical analysis of foods. London, J. and A. Churchill, 6th ed. 1970
- Schmidt, P.J. and D.R. Idler, Predicting the colour of canned sock-eye salmon from the colour of the raw flesh. Food Technol., 12:44-8
- Stansby, M.E. and J. Desson, Use of frozen salmon for canning. Commer.Fish.Rev., 13(4):20-5 1957
- Tenikave, E. and T. Motohiro, Freezing of salmon for canning. Bull.Int.Inst.Refrig., 39:870 1959
- Tanikawa, E., T. Motohiro and I. Sheji, Studies on the manufacture of canned salmon. The odour in canned salmon manufactured from frozen fish raw material. Bull.Fac.Fish. Hokkaido Univ., (6):317-40
- Van den Broek, C.J.H., Fish canning. In Fish as food, edited by G. Borgstrom. New York, Academic Press, Vol.4:127-203
- Vyncke, W., Evaluation of direct thiobarbituric acid extraction for the determination of oxidative rancidity in mackerel (*Scomber scombrus*). Paper presented in the meeting of the Joint FAO/WHO Expert Committee on Fish and Shellfish Hygiene, Geneva

THE HYGIENE STATUS OF SEAFOOD IN MELBOURNE

by

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ABSTRACT

A survey of seafoods (80): scallops, mussels, oysters and "wet" fish at retail outlets showed that the temperature at the time of purchase ranged from -3 to 16°C. Of the seafoods tested, 54% had total bacterial counts between 10^3 and 10^6 /g and 97% had *Staphylococcus aureus* count of less than 10^2 /g. Coliform counts of 81% of the samples were less than 10^2 /g and faecal coliform counts of 91% of the samples were less than 10/g. *Enterobacteriaceae* and *Escherichia coli* count of 76% of the samples were less than 10^4 /g and less than 10/g, respectively. The greatest number of *Vibrio parahaemolyticus* obtained was 4/g in 25% of the samples tested. The number of hydrogen sulphide (H_2S) producers ranged from less than 10^2 /g to 10^6 /g, with 42 of 80 (53%) samples having a H_2S count representing less than 10% of the total count.

1. INTRODUCTION

Millions of tons, for example, 72 191 000 tons (FAO, 1980) of marine fish and shellfish are consumed annually throughout the world and it is hardly surprising that food poisoning should sometimes occur (Barrow, 1973). However, the incidents of foodborne outbreaks due to seafood were low in comparison to outbreaks due to meat and poultry. Todd (1978) reported that in Australia (1967-71), USA, Canada, England and Wales (1973-75), the percentage of foodborne outbreaks due to fish and shellfish were 12.5, 9.3, 5.8 and 0.5%, respectively. However, data on food poisoning were only recorded from countries with surveillance programmes, that is, developed countries and no data was available from developing countries. The figures for incidents alone can be misleading unless the number of cases are considered also.

For example, in the food poisoning incidents which occurred in the USA (1972) and UK (1972), 500 and 700 people were involved, respectively. The source of infection from a local restaurant in Paragould, USA, was traced back to Peruvian fish meal contaminated with *Salmonella agona*. One outbreak involved 600 people who became ill with acute gastroenteritis after attending a "shrimp boil" party in Louisiana and the second outbreak involved 27 people who ate shrimp and crabs in New Jersey (Anon., 1972). More recently, from 1979 to 1983 in Florida, USA, 34 people became ill after consuming raw oysters contaminated with *Vibrio* spp. (Wood, Baker and Singleton, 1984). A shigellosis outbreak in Holland in early 1984, caused 14 deaths and 120 cases of discomfort due to contaminated shrimp (James, 1984).

In Australia, due to a high consumption of crustaceans and molluscs, that is 83 528 tons in 1982-83 (Cameron, 1983) and with constant increase in the pollution of rivers and estuaries, the risk of oysters, mussels and other seafoods becoming contaminated with food poisoning organisms is increasing (Sutton, 1973). One such nationwide outbreak (1978) occurred where 2 000 people were affected in an oyster associated food poisoning due to Norwalk virus (Murphy *et al.*, 1979). By 1979, another 61 public servants were ill after tasting the oysters (Christopher, Murphy and Grohman, 1980).

According to Hobbs (1973), microbiological standards for viable counts and index organisms provided useful guidelines for cleanliness of food and safety in public health. Barrow (1973) stated that good bacterial standards in seafoods were dependent on hygienic handling and plant sanitation. Barrow (1973) also believed that safety, however, ultimately depended not so much on microbiological standards as on education in hygiene and local eating habits.

In Victoria, neither the handling procedure nor the hygiene status of seafoods have been investigated. Hence, a survey was conducted to assess the hygiene status of retail seafoods in

Melbourne. In the survey, indicator organisms, total bacterial count, coliforms, *Enterobacteriaceae* organisms of public health significance such as *Staphylococcus aureus*, *Escherichia coli* and *Vibrio parahaemolyticus* and spoilage organisms such as hydrogen sulphide producers were enumerated.

2. MATERIALS AND METHODS

In 1984, 80 samples of seafoods were purchased, 24 oysters, 23 fish, 21 scallops and 12 mussels at retail outlets in Melbourne. Temperatures of the samples were recorded on purchase and samples were transported to the laboratory in an insulated container. Samples were stored in a refrigerator and analysed within 2-3 h of purchase.

A decimal dilution of each sample (20-25 g) with peptone water (0.1% w/v) was macerated in a Colworth Stomacher for 60 sec. Serial dilutions in peptone water (0.1% w/v) were added to the surface of duplicate nutrient agar (Oxoid CM 3) plates, which were incubated at 20°C for 4 days. Plates containing 30-300 colonies were counted, the counts averaged and reported as total bacterial count/g. *Enterobacteriaceae* count enumerated by using surface inoculated plates of violet red bile glucose agar (Oxoid CM 485) and incubated at 37°C for 48 h. *Escherichia coli* count was enumerated by the Anderson-Baird Parker method (1975) using tryptone bile agar (TBA, Oxoid CM 595). Coliform count and faecal coliform count enumerated by the most probable number (MPN) methods as in Australian Standard Method 1766, Part 2-1976, Section 1.3.5. *Staphylococcus aureus* was enumerated using spread drop plates of Baird Parker medium (Oxoid CM 275). *Vibrio parahaemolyticus* was enumerated by the Australian Standard 1766, Part 2-1976, Section 1.9.4. Hydrogen sulphide producers enumerated by using drop plates of iron agar (modified from Levin, 1968) and incubated at 20°C for 4 days.

3. RESULTS

Temperatures of 80 samples purchased ranged from 4 to 16°C where 53 samples (66%) had temperatures of less than 4°C and 17 samples (21%) had recorded temperature up to 16°C (Table 2). Total bacterial count of samples ranged from 10^3 - 10^8 /g. Of 56 fish, scallops and mussels, 44 (78%) samples had total bacterial fish, scallops and mussels, 44 (78%) samples had total bacterial count of 10^5 - 10^7 /g (Table 3) while 5 samples (6%) had total bacterial count of 10^1 - 10^8 /g. Oysters (50%) had counts of one log scale lower, that is, 10^2 - 10^6 /g. *Staphylococcus aureus* counts of less than 10^1 /g was found in 70 (97%) of 72 seafoods tested (Table 4).

Coliform count (Table 5) showed that 35 (81%) samples out of 43 had counts less than 10^2 /g while 43 (97%) samples out of 44 tested had faecal coliform counts of less than 10^2 /g (Table 6). Of 79 samples, 9 (11%) had *Escherichia coli* counts greater than 10^1 /g (Table 7). For *Enterobacteriaceae* counts, 9 (11%) samples in 80 tested had counts that ranged from 10^2 - 10^6 /g (Table 8). In 15 tests *Vibrio parahaemolyticus*, enumerated using GSTB as enrichment broth and subsequently streaked onto TCBS, had a maximum count of 4/g. The number of hydrogen sulphide (H_2S) producers, regarded as potential spoilers (Chai *et al.*, 1968), in retail samples ranged from 10^1 to 10^4 /g (Table 9). Hydrogen sulphide producers represented 10-30% of total bacterial count in 17 (21%) of 80 samples tested (Table 10).

4. DISCUSSION

For fish surveyed (240) in the Netherlands (Ven den Broek, Mossel and Mol, 1984), like those examined in the present survey, approximately 50% of fish samples had total bacterial counts in the range 10^6 - 10^7 /g. However, a higher percentage of fish samples in the present survey, exceeded the *Escherichia coli* count of less than 10^1 /g, that is, 17% in comparison to 7% of samples in the Netherlands.

The majority (> 50%) of oysters tested in the USA (Foster, Fowler and Dacey, 1977) survey had total bacterial count of one log scale higher, at 10^7 - 10^8 /g, then the majority (> 50%) of samples in the present survey. However, no oysters in the USA study of 59 oysters had *Escherichia coli* count in excess of 10^1 /g whereas the present survey found that 13% of oysters tested exceeded the limit.

Scallops surveyed (51) in the USA (Foster, Fowler and Dacey, 1977) had 63% of scallops with total bacterial count of 10^7 - 10^8 /g compared to the 9% of scallops in the present survey with total bacterial count in that range. However, 25% of scallops in the present survey exceeded the *Escherichia coli* limit of less than 10^1 /g whereas only 2% of scallops in USA studies exceeded the limit. *Staphylococcus aureus* count was comparable in all surveys.

Overall, although the total bacterial counts in the present survey were 2 log scales than other surveys (Andrews *et al.*, 1977; Foster, Fowler and Dacey, 1977; Van den Broek, Mossel and Mol, 1984), the percentage of samples that had *Escherichia coli* counts in excess of 10^1 /g was greater in the present survey. Hence, the above data shows that total bacteria count should not be used as a measure of safety (Silliker, 1973) because of poor correlation between total bacterial count and *Escherichia coli* count.

The results from the survey were compared with the microbiological standards recommended by the International Commission of Microbiological Standards of Foods (ICMSF, 1974). The Commission recommended that raw fish product having total bacterial count of less than 10^6 /g, should be considered good quality while those having total bacterial counts in excess of 10^7 /g should be considered unacceptable. Of 80 seafoods tested, 33 (40%) exceeded the ICMSF recommended limit for a good quality product. However, only 6.3% of seafood tested were not acceptable by ICMSF recommended limit of 10^6 /g.

Organisms such as coliforms, faecal coliforms, *Escherichia coli* and *Enterobacteriaceae* were used as indication of faecal pollution (Hughes, Merson and Gangarosa, 1977; Matches and Abeyta, 1983) in the water where the shellfish were harvested. Of 43 samples tested, 8 (19%) samples would not have complied to the suggested guideline by Shevan (1970) for coliform count of less than 10^2 /g, an indication of faecal contamination.

The ICMSF recommended a limit of 4×10^2 /g for both faecal coliforms and *Escherichia coli*. Under this standard, 1 sample had faecal coliform count and 5 samples had *Escherichia coli* count greater than the limit. Out of 80 samples tested, 48 (60%) had high *Enterobacteriaceae* count of 10^5 /g. Since only a small percentage (6%) of samples had *Escherichia coli* count in excess of 4×10^2 /g (ICMSF), the high percentage of samples with *Enterobacteriaceae* count of 10^5 /g would have considered of non-pathogenic coliforms.

The recommended limit by ICMSF for *Staphylococcus aureus* value is 10^3 /g. Of the seafoods tested, 100% of the samples complied to the guideline. The present study found only low (4/g) numbers of *Vibrio parahaemolyticus* in seafoods. If food is adequately cooked or refrigerated, multiplication of organisms in foods can be controlled (Desmarchelier, 1978).

Of the 80 samples tested, 17 (21%) samples had total bacterial count of which 10-30% were H_2S producers. There were 21 samples (26%) in which H_2S producers represented greater than 30% of total bacterial count. At such high levels the presence of H_2S producers signified spoilage (Chai *et al.*, 1968). Since there were 27 (34%) samples held at 5-16°C at time of purchase, it was not surprising to find samples with H_2S producers which represented a high percentage of total bacterial count.

The high percentage (47%) of samples had high ratios (10% to >30%) of H_2S producers to total bacterial count indicated temperature abuse of seafoods. Although seafoods were subjected to temperature abuse, very few numbers of *Vibrio parahaemolyticus* were enumerated (maximum 4/g). Further, contamination either by handling or faecal has been minimal because no samples exceeded the ICMSF *Staphylococcus aureus* limits and very few samples (6%) exceeded the ICMSF *Escherichia coli* limit.

5. REFERENCES

- Anderson, J.M. and A.C. Baird-Parker, A rapid and direct plate method for enumerating *Escherichia coli* biotype 1 in food. *J. Appl. Bacteriol.*, 39:111-7
- Andrews, W.H., *et al.*, Bacteriological survey of the channel catfish (*Ictalurus punctatus*) at the retail level. *J. Food Sci.*, 42:359-63
- Barrow, G.I., Marine micro-organisms and food poisoning. In *Microbiological safety of food*, edited 1973 by B.C. Hobbs and J.H.B. Christian. London, Academic Press
- Cameron, R.J., Apparent consumption of foodstuffs and nutrients, Australia. Canberra, Australian 1983 Bureau of Statistics
- Chai, T., *et al.*, Detection and incidence of specific species of spoilage bacteria on fish. 1968 *Appl. Microbiol.*, 16:1738-41
- Christopher, P.J., A.M. Murphy and G.S. Grohmen, Oyster testers and Norwalk virus. *N.Z. J. Med.*, 1980 10:458-63
- Desmarchelier, P., *Vibrio parahaemolyticus* and other vibrios. *Food Technol., Aust.*, 30:339-45 1978
- FAO, Yearbook of fishery statistics. Food and Agriculture Organization of the United Nations - 1980 Rome, 50:39
- Foster, J.F., J.L. Fowler and J. Dacey, A microbial survey of various fresh and frozen seafood products. *J. Food Prot.*, 40:300-3
- Hobbs, S.C., Food poisoning in England and Wales. In *Microbiological safety of food*, edited by 1973 B.C. Hobbs and J.H.B. Christian. London, Academic Press

- Hood, M.A., R.M. Baker and F.L. Singleton, Effect of processing and storing oyster meats on concentrations of indicator bacteria, vibrios and *Aeromonas hydrophila*. J.Food Prot., 1984 47:598-601
- Hughes, J.M., M.H. Merson and E.J. Gangarosa, The safety of eating shellfish. Jama, 237:1980-1 1977
- ICMSF (International Commission on Microbiological Specification for Foods), Microorganisms in foods. 2. Sampling for microbiological analysis: principles and specific applications. Toronto, Canada, University of Toronto Press, pp. 92-104
- James, D., Food poisoning in Holland. Fish Tech.News, 7 1984
- Lavin, R.E., Detection and incidence of specific species of spoilage bacteria on fish. 1. Methodology. Appl.Microbiol., 16:1734-7 1968
- Matchas, J.R. and C. Abayta, Indicator organisms in fish and shellfish. Food Technol., 37:114-7 1983
- Murphy, A.M., et al., An Australian-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. Med.J.Aust., 2:329-33 1979
- Shewan, J.M., Bacteriological standards for fish and fishery products. Chem.Ind., 6:193-9 1970
- Silliker, J.H., Total counts as indexes of food quality. In Microbiological quality of foods, edited by L.W. Slanetz et al., New York, Academic Press, 273 p. 1973
- Standards Association of Australia, Australian Standard 1766. Methods for the microbiological examination of food. Sydney, Standards Association of Australia 1976
- Sutton, R.G.A., Food poisoning and Salmonella infections in Australia. In Microbiological safety of food, edited by B.C. Hobbs and J.N.B. Christian, London, Academic Press 1973
- Todd, E.C.D., Foodborne diseases in six countries - a comparison. J.Food Prot., 41:559-65 1978
- Van der Broek, M.J.M., D.A.A. Mossal and H. Mol, Microbiological quality of retail fish fillets in the Netherlands. Int.J.Food Microbiol., 1:53-61 1984
- Anon., US Centre for Disease Control. Morbid.Mortal.Weekly Rep., (21):67 1972

Table 1

Tabulation of retail seafoods purchased

Type of samples	Common name	Scientific name	No. tasted	Total no.
Oysters	Oysters	<u>Crassostrea virginica</u>	24	24
		<u>Ostrea edulis</u>		
Scallops	Scallops	<u>Chlamys opercularia</u>	21	21
Mussels	Mussels	<u>Mytilus edulis</u>	12	12
Fish	Rainbow trout	<u>Salmo gairdneri</u>	5	23
	John Dory	<u>Zeus tater</u>	1	
	King George Whiting	<u>Sillaginodes punctatus</u>	1	
	Snapper	<u>Chrysophrys auratus</u>	6	
	Flounder	<u>Rhomboclelea tapirina</u>	3	
	Bream	<u>Acanthopagrus butcheri</u>	5	
	Garfish	<u>Hemiramphus melanochir</u>	2	

Table 2

Temperature of samples at time of purchase

Temperature (°C)	No. (%) of samples from		
	Victoria market	Retail shops	Supermarkets
-3 to 0	-	28 (61)	11 (79)
1 to 4	3 (15)	11 (24)	-
5 to 8	11 (55)	5 (11)	-
9 to 16	6 (30)	2 (4)	3 (21)
Total number tasted	20 (100)	46 (100)	14 (100)

Table 3

Total bacterial count of retail seafood purchased

Count / g *	Number (%) of				Total no. (%)
	Oysters	Fish	Scallops	Mussels	
$10^3 - 10^4$	6 (25)	0	0	0	6 (8)
$10^4 - 10^5$	1 (4)	2 (9)	4 (19)	1 (8)	8 (10)
$10^5 - 10^6$	12 (50)	9 (39)	5 (24)	3 (25)	29 (36)
$10^6 - 10^7$	5 (21)	10 (43)	10 (48)	7 (58)	32 (40)
$10^7 - 10^8$	0	2 (9)	2 (9)	1 (8)	5 (6)
Total number tested	24 (100)	23 (100)	21 (100)	12 (100)	80 (100)

* Total bacteria count on nutrient agar (Oxoid CM3) incubated at 20°C for 4 days

Table 4

Staphylococcus aureus count of retail seafoods purchased

Count / g *	Number (%) of				Total no. (%)
	Oysters	Fish	Scallops	Mussels	
< 10	13 (72)	14 (61)	13 (62)	7 (70)	47 (65)
$10^1 - 10^2$	4 (22)	9 (39)	7 (33)	3 (30)	23 (32)
$10^2 - 10^3$	1 (6)	0	1 (5)	0	2 (3)

* *Staphylococcus aureus* enumerated on Baird - Parker agar (Oxoid CM 275) incubated at 37°C for 48 h

Table 5

Coliform count of retail seafoods purchased

Count / g *	Number (%) of				Total no. (%)
	Oysters	Fish	Scallops	Mussels	
< 10	2 (25)	6 (33)	8 (73)	4 (67)	20 (47)
$10^1 - 10^2$	5 (62)	7 (39)	2 (18)	1 (17)	15 (35)
$10^2 - 10^3$	0	4 (22)	0	0	4 (9)
$10^3 - 10^4$	1 (13)	1 (5)	1 (9)	1 (17)	4 (9)

* Coliform count enumerated by method using Australian Standard method 1766 Part 2 - 1976, section 1:3.5

Table 6

Faecal coliform count of retail seafoods purchased

Count / g *	Number (%) of				Total no. (%)
	Oysters	Fish	Scallops	Mussels	
< 10	7 (88)	15 (83)	11 (92)	6 (100)	39 (91)
$10^1 - 10^2$	1 (12)	2 (11)	1 (8)	0	3 (7)
$10^2 - 10^3$	0	1 (6)	0	0	1 (2)

* Faecal coliform count enumerated by MPN method using Australian Standard method 1766 Part 2 - 1976, section 1:3.5

Table 7

Escherichia coli 1 count of retail seafoods purchased

Count / g *	Number (#) purchased				Total no. (#)
	Oysters	Fish	Scallops	Mussels	
< 10	20 (83)	19 (83)	16 (76)	8 (67)	63 (80)
$10^1 - 10^2$	0	3 (13)	2 (9)	2 (16)	7 (9)
$10^2 - 10^3$	1 (4)	1 (4)	2 (9)	1 (8)	5 (6)
$10^3 - 10^4$	2 (9)	0	1 (5)	1 (8)	4 (5)

* *Escherichia coli* 1 enumerated by Anderson - Baird Parker method (1975)

Table 8

Enterobacteriaceae count of retail seafoods purchased

Count / g *	Number (#) of				Total no. (#)
	Oysters	Fish	Scallops	Mussels	
< 10	1 (4)	1 (4)	1 (5)	0	3 (4)
$10^1 - 10^3$	6 (25)	5 (22)	4 (18)	5 (41)	20 (25)
$10^3 - 10^5$	13 (54)	17 (74)	11 (52)	7 (58)	48 (60)
$10^5 - 10^6$	4 (17)	0	5 (24)	0	9 (11)

* Enterobacteriaceae count on violet red bile glucose agar (Oxoid CM485) incubated at 37°C for 48 h

Table 9

Number of hydrogen sulphide producers in retail seafoods purchased

Count / g *	Number (%) of				Total no. (%)
	Oysters	Fish	Scallops	Mussels	
$< 10^2$	6 (25)	0	1 (5)	0	7 (9)
$10^2 - 10^4$	5 (21)	4 (17)	6 (29)	4 (33)	19 (24)
$10^4 - 10^6$	10 (42)	11 (61)	9 (43)	6 (50)	36 (45)
$10^6 - 10^8$	3 (12)	5 (21)	5 (24)	2 (17)	15 (22)

* Hydrogen sulphide producers enumerated on iron agar (modified from Levin 1968) incubated at 20°C for 4 days

Table 10

Hydrogen sulphide producers (as a percentage of total bacterial count) in retail seafoods purchased

% H ₂ S *	Number (%) of				Total no. (%)
	Oysters	Fish	Scallops	Mussels	
< 10	14 (58)	3 (39)	10 (48)	9 (76)	42 (53)
$10 - 30$	5 (21)	6 (26)	4 (19)	2 (16)	17 (21)
> 30	5 (21)	8 (35)	7 (33)	1 (8)	21 (26)

* Hydrogen sulphide producers as a percentage of total bacterial count

MICROBIOLOGICAL QUALITY OF RETAIL "WET" FISH
IN JAKARTA

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ABSTRACT

Microbiological and sensory quality of 73 samples of retail fish (9 species) were examined. Of the commercial fish tested, 30% had a raw quality score of 16-20. The temperature of the fish at the time of purchase ranged from 17°C to 25°C and the total bacterial count (30°C) ranged from 10^3 to 10^8 /g with 72% of the samples tested having counts between 10^6 to 10^8 /g. A count on violet-red bile glucose agar (30°C) and iron agar (H_2S) producers of between 10^5 to 10^7 /g was obtained for 63% and 56% of the samples, respectively.

1. INTRODUCTION

Although Indonesia is among the world's major fishing nations (11th place in 1980), harvesting 18 500 t (FAO, 1981) in 1980, a 50% increase compared to 1971, little is known of the quality of fish bought by consumers at retail markets. The participants of a collaborative project (No. 8304), "Tradition and control of the spoilage of fresh, cured and dried tropical fish", sponsored by the Agency for Agricultural Research and Development (AARD) in Indonesia and the Australian Centre for International Agricultural Research (ACIAR), are involved in collecting data on aspects of the fishing industry (of West Java) from catch to consumption. As part of the collaborative project, a study was made of the handling, quality (visual and microbiological) and value of the fish at retail markets in Jakarta.

2. MATERIALS AND METHODS

In 1983-84, 73 fish samples, listed in Table 1, were purchased at retail markets: Senen, Palmerah, Tanah Abang, Ben Hilir and others in Jakarta. Temperature measurements were made by inserting a mercury thermometer into the anus (approximately 0.5 cm) of each fish just after purchase, and the temperature recorded. Fish were transported, as packaged, to the laboratory and held at ambient (28°-30°C) until tested, 2-3 h after purchase.

Fish flesh (10 g) of the belly flap region was aseptically diluted with peptone water (0.1% w/v) and homogenized in a Waring blender for 60 sec. Aliquots of serial dilutions in peptone water (0.1% w/v) were added to the surface of duplicate nutrient agar (Oxoid CM 3) plates for total bacterial counts, violet-red bile glucose agar (VREBA, Oxoid, CM 485) plates for *Enterobacteriaceae* counts and iron agar (Table 2) for enumerating hydrogen sulphide producing bacteria as black colonies. Plates were incubated at 30°C for three days and colonies counted, averaged and reported as counts per gramme.

Organoleptically, the fish were evaluated by the demerit score system devised by Olley (pars. comm., 1983). The demerit score (Table 3) for raw quality ranges from zero for pre-rigor "wet" fish to a maximum of 39 for spoiled "wet" fish. Further, fish recording a demerit score of less than 5, <18, <24 or greater than 24 was graded as prime, good, fair and poor quality, respectively.

3. RESULTS AND DISCUSSION

3.1 Temperature Measurement

The temperature of fish at the time of purchase ranged from 17° to 25°C with 57 (78%) of samples registering a temperature greater than 20°C (Table 4). By contrast, Sumner, Orajana and Hipol-Estrada (1984) recorded the temperature of 60 fish at Cubao Farmer's market in the Philippines, which ranged from 5° to 23°C, with a mean of 16.5°C.

A check of stalls at retail markets in Jakarta revealed that no stalls were using ice. Thus, ice usage was certainly not related to price since neither fish priced at Rp 500/kg, such as milk-

fish (*Chanos chanos*) nor fish costing Rp 2 500/kg, such as barramundi (*Lates calcarifer*) were iced, whereas at the Cubao Farmer's market Sumner, Orejana and Nipol-Estrada (1984) found that 20/78 stalls used ice. Interestingly, usage was again independent of value of produce.

3.2 Sensory Evaluation

Fish purchased at retail markets in Jakarta had demerit (raw) scores (Table 5) ranging from 5 to 25, where 25 represented 64% of the maximum possible score (of 39). According to the demerit scoring sheet, 72 (99%) of the fish purchased were of fair quality. Furthermore, of these (99%), 17 (24%) fish were approaching poor (a score of greater than 24) quality with a score of 16 to 20.

Interestingly, mullet (3 pieces) had a raw quality score at rejection of 31 to 34 after 21 days in ice (Saleh *et al.*, these proceedings). Thus, 24% of the fish purchased at retail markets in Jakarta were equivalent to 13 days, 2/3 of the shelf life (based on mullet study) in ice. However, the relationship between demerit score used in the present study and days in ice for a range of tropical fish has not been established.

3.3 Microbiological Analysis

3.3.1 Total bacterial count

Total bacterial count on NA incubated at 30°C for three days ranged from 10^3 to 10^8 /g for fish purchased at retail outlets in Jakarta (Table 6). The majority (72%) had total bacterial counts of 10^6 - 10^8 /g.

Interestingly, Barila *et al.* (these proceedings) found that Faughn's mackerel (*Rastrelliger faughni*, Matsui), iced after 0 h, 3 h, 6 h, 9 h and 12 h at ambient (28°-30°C) in the Philippines, had total bacterial counts (20°C) at rejection of 10^3 , 10^7 , 10^6 and 10^5 /g, respectively. In another study, Barila *et al.* (these proceedings) showed that when fish (*Rastrelliger faughni*, Matsui) were rejected, the total bacterial count was 10^7 /g, whether the fish was stored at 0, 5, 10 °C or at ambient temperature (28°-30°C). The retail fish in Jakarta, held at ambient temperature (17°-25°C) had total bacterial counts in excess of 10^7 /g in 15 (28%) fish at the time of purchase.

3.3.2 Hydrogen sulphide (H_2S) producing bacteria

Hydrogen sulphide (H_2S) producing bacteria, identified as black colonies on iron agar (modified Levin's iron agar, 1968), ranged from 10^3 to 10^8 /g on fish bought at retail outlets in Jakarta. At rejection, the number of H_2S producing bacteria reached 10^7 /g for fish stored in ice (Chai *et al.*, 1968; Shewan, 1977 and Gorczyca, 1983). By contrast, at ambient (28°-35°C) storage, the number of H_2S producing bacteria recorded at rejection was generally 1 log scale lower, at 10^6 /g (Estrada *et al.* and Saleh *et al.*, these proceedings; and Gorczyca *et al.*, 1985). Of 42 fish tested, 29 (46%) had counts of H_2S producers in the range of 10^6 - 10^7 /g (Table 7).

Significantly, H_2S producing bacteria, identified as *Pseudomonas* (*Aeromonas putrefaciens*, Shewan, 1977), have been associated with spoilage of fish (tropical and temperate) stored in ice (chilled) because firstly, the number of H_2S producers increased during storage significantly; from less than 1% initially to 10-20% of the total bacterial count at rejection (Chai *et al.*, 1968; Gorczyca, 1983). Secondly, many workers have determined the biochemical activity of *Aeromonas putrefaciens* in producing spoilage by-products, such as trimethylamine, volatile sulphides other than H_2S and nucleotide by-products: inosine and hypoxanthine (Chai *et al.*, 1968; Herbert *et al.*, 1971; Shewan, 1977; Van Spreeken, 1977). However, no association, as yet, has been established between H_2S producing bacteria and spoilage of fish at tropical temperatures (28°-35°C).

3.3.4 Enterobacteriaceae count

The count of bright purple colonies with halos, that is presumptive Enterobacteriaceae, on violet-red bile glucose agar (VRBA), ranged from 10^3 to 10^8 /g for fish bought at retail markets in Jakarta. Of the fish purchased, 13 (24%) had Enterobacteriaceae counts of 10^6 - 10^7 /g (Table 8).

Significantly, several studies (Estrada *et al.*; Gorczyca and Pek Poh Len, these proceedings; Gorczyca *et al.*, 1985), involving storage of fish (freshwater and marine) at tropical temperatures (28°-35°C), found that counts on VRBA increased to 10^7 - 10^8 /g at rejection. Furthermore, the microflora isolated from VRBA, namely *Aeromonas* spp., *Enterobacter* spp. and *Citrobacter* spp., were shown to be biochemically active in producing spoilage by-products, for example, trimethylamine and volatile sulphides (other than H_2S) and "off-odours" on sterile fish muscle. Thus, counts on VRBA may have potential, not only indicator of health hazard (faecal contamination-*Escherichia coli* Type 1 as Enterobacteriaceae, but also as an indicator of spoilage.

Overall, the present study has shown that the quality (as judged visually and microbiologically) of "wet" fish at retail outlets in Jakarta, ranged from fair (25%) to good (74%). Furthermore, temperature control was not being practised at the retail level as no icing of fish was observed.

Loss of quality has often been associated with poor temperature control. The advantages of improving fish quality according to Sumner, Orejana and Hipol-Estrada (1984) are: "firstly, public health aspects might be improved; secondly, that the consumer would prefer better quality and would buy more fish or pay a better price; thirdly, that spoilage would be reduced".

Regretably, the present paper has only dealt with the final segment of the fishing industry, the retail outlet. Data needs to be gathered on aspects of fish handling, value, quality and quantity from catch to retailing before recommendations on upgrading quality at any one sector of the fishing industry can be proposed.

4. REFERENCES

- Chai, T., et al., Detection and incidence of specific species of spoilage bacteria on fish. 2. 1968 Relative incidence of *Pseudomonas putrefaciens* and fluorescent *Pseudomonas* on haddock filets. Appl.Microbiol., 16:1738-41
- FAO, Yearbook of fishery statistics. *Annuaire statistique des pêches. Anuario estadístico da* 1981 *pesca. Catches and landings. Captures et quantités débarquées. Capturas y* desembarques. 1980. Yearb.Fish.Stat./Annu.Stat.Pêches/Anu.Estad.Pesca, (50):386 p.
- Gorczyca, E.M., Studies on the shelf life extensions of retail fish filets. M.Appl.Sci. Thesis. 1983 RMIT, Melbourne, Australia
- Gorczyca, E.M., et al., Mesophilic fish spoilage. Food Technol., Aust., 37 1985
- Herbert, R.A., et al., Bacteria active in the spoilage of certain seafoods. J.Appl.Bacteriol., 1971 34:41-50
- Levin, R.E., Detection and incidence of specific species of spoilage bacteria on fish. 1. 1968 Methodology. Appl.Microbiol., 16:1734-7
- Shewan, J.M., The bacteriology of fresh and spoiling fish and the biochemical changes induced by 1977 bacterial action. In Proceedings of the Conference on handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 51-66
- Sumner, J., F.M. Orejana and M. Hipol-Estrada, The quality of retail wet fish in the Philippines: 1984 some observations on the effect of on-board and on-land handling. Canberra, ACIAR (Australian Centre for International Agricultural Research) Report for project No. 8304
- Van Sprekens, K.J., Characterisation of some fish and shrimp spoiling bacteria. Antonie van 1977 Leeuwenhoek, 43:283-303

Table 1

Fish species bought at retail markets

Fish Species			
Scientific name	Indonesian	English	Number tested
<u>Rastrelliger</u> sp.	Kembung	Mackerel (chub)	9
<u>Auxis</u> <u>thazard</u>	Tongkol	Mackerel (frigate)	6
<u>Scomberomorus</u> sp.	Tengiri	Mackerel (spanish)	8
<u>Chanos</u> <u>chanos</u>	Bandeng	Milkfish	10
<u>Mugil</u> <u>cephalus</u>	Belanak	Mullet	8
<u>Formia</u> <u>niger</u>	Bawal hitam	Pomfret (black)	10
<u>Katsuwonus</u> <u>pelamis</u>	Cakalang	Skipjack tuna	5
<u>Lutjanus</u> <u>sanguineus</u>	Ikan merah	Snapper	9
<u>Selaroides</u> <u>leptolepis</u>	Selar kuning	Trevally (yellow stripe)	8

Table 2

Modified iron agar (Levin, 1968)

Ingredients	Quantity (g/L)
Beef extract	3.0
Yeast extract	3.0
Peptone	5.6
Tryptone	15.0
Ferric citrate	0.3
Sodium thiosulphate	0.5
Sodium chloride	5.0
Agar	12.0

The pH of the basal medium was adjusted to 7.2 + 0.1 using sodium hydroxide pellets (85% purity). The medium was sterilized at 121°C for 15 min.

Aseptically, 10 ml of separately autoclaved cysteine solution (0.04% w/v) was added to the basal medium (1L).

A cold overlay of peptone-iron agar was placed onto the peptone-iron agar plate as follows: molten peptone-iron agar was poured into rings (9 cm diam.) in a laminar flow cabinet (Clemco, Australia).

After setting, the agar discs were manipulated aseptically onto inoculated peptone-iron agar plate.

After incubation at 30°C for three days, black colonies were counted, and expressed as H₂S producing bacteria/gramme of fish.

Name : _____
Date : _____

[illegible]

0	V. bright (sangat mengkilap)								
1	Bright (mengkilap)								
2	Sl. dull (agak kusam)								
3	Dull (kusam)								

0	Firm (kuat)							
1	Soft (lunak)							

0	Firm (kuat)						
1	Sl. loose (agak mudah lepas)						
2	Loose (mudah lepas)						

0	Absent (tidak ada)								
1	Sl. slimy (agak berlendir)								
2	Slimy (berlendir)								
3	V. slimy (sangat berlendir)								

0	Pre-rigor								
1	Rigor								
2	Post rigor								

0	Clear (cerah)						
1	Sl. cloudy (agak berkabut)						
2	Cloudy (berkabut)						

0	Normal						
1	Sl. sunken (agak tenggelam)						
2	Sunken (tenggelam)						

0	Visible (terlihat)						
1	Not visible (tidak terlihat)						

0	No blood (tidak terlihat)							
1	Sl. bloody (sedikit berdarah)							
2	V. bloody (sangat banyak darah)							

Table 4

Temperature of retail fish at time of purchase^{a/}

Temperature (°C)	Number of fish
17 - 20	16 (22%)
20 - 22	24 (33%)
23 - 25	33 (45%)
Total	73 (100%)

^{a/} Time of purchase at retail stores in Jakarta was 8 a.m.

Table 5

Raw quality of retail fish in Jakarta (1983-84)

Range of raw scores	Number of fish
0 - 4	0 (0%)
5 - 10	18 (25%)
11 - 15	37 (50%)
16 - 20	17 (24%)
21 - 25	1 (1%)
26 - 39	0 (0%)
Total	73 (100%)

Table 6

Microbiological quality of fish brought at retail markets in Jakarta (1983-84)

Range of bacterial count on NA after incubation at 30°C for three days	Number of fish
$10^3 - 10^5$	4 (8%)
$10^5 - 10^6$	11 (20%)
$10^6 - 10^7$	24 (44%)
$10^7 - 10^8$	15 (28%)

Table 7

Microbiological quality of retail fish in Jakarta (1983-84)

Range of H_2S producers on iron agar after incubation at 30°C for three days	Number of fish
$10^3 - 10^5$	2 (3%)
$10^5 - 10^6$	6 (10%)
$10^6 - 10^7$	29 (46%)
$10^7 - 10^8$	5 (8%)

Table 8

Microbiological quality of retail fish in Jakarta (1983-84)

Range of Enterobacteriaceae count on VRBGA after incubation at 30°C for three days	Number of fish
$10^3 - 10^4$	6 (11%)
$10^4 - 10^5$	14 (26%)
$10^5 - 10^6$	20 (37%)
$10^6 - 10^7$	13 (24%)
$10^7 - 10^8$	1 (2%)

IMPROVEMENT OF QUALITY AND YIELDS OF TROPICAL FISH

by

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ABSTRACT

There have been reports in the literature of a cold shock reaction in certain species of fish, particularly those from tropical waters, when they are iced soon after death. This paper reviews the work which has been carried out on this phenomenon. Tropical fish which exhibit the cold shock reaction stiffen within minutes of being placed in ice. This appears to be accompanied initially by an accelerated metabolism which is superficially similar to that of mammalian muscle which has undergone cold shortening. Objective measurement of muscle tension, however, has shown that, unlike cold-shortened muscles, fish muscle fibres do not contract during the cold shock reaction. It has been found that this reaction significantly affects fillet yields and quality. The practical implications of these results for tropical fisheries are discussed.

1. INTRODUCTION

Although the affect of handling and processing on the quality and yields of cold and temperate water fish has been extensively studied, in comparison very little work has been carried out on tropical species. As a consequence, technologists have had to rely upon the experience and established practices in coldwater fisheries and the FAO/WHO International Codes of Practice are based on this knowledge. In view of the fact that it has been known for some time (Disney, Cole and Jones, 1974) that tropical species do not always react to handling and processing in the same manner as fish from colder waters, this has raised some concern among technologists working in warmwater fisheries. This has become particularly important in recent years as improved and industrial processes have been applied to tropical species.

The relevance of the existing codes of practice to tropical conditions has been reviewed by the former Tropical Products Institute (now the Tropical Development and Research Institute) for FAO (TPI, 1975). In addition, a number of areas have been identified where important differences exist between species from tropical and cold waters (Poulter, Curran and Disney, 1981). This paper reviews the work, to date, on one of these differences: that of the cold shock reaction reported in some tropical species.

2. COLD SHOCK REACTION IN TROPICAL FISH

Although the contraction of muscle following death known as *rigor mortis* also affects meat and poultry processing, the problems which it causes for the fishing industry may be more acute (Stroud, 1969). It has been claimed that it affects the post-mortem autolytic and bacterial spoilage of fish flesh (Lovatt, 1952) and even predetermines the shelf life of the fresh product (Amlacher, 1961). Handling of coldwater fish during rigor can result in a loss of quality and a lower yield (Bramsen and Hansen, 1965).

The development and resolution of *rigor mortis* depend upon a variety of factors: species, physiological condition, size, amount of handling during rigor and temperature. Commercially, the last of these, temperature, is considered to be the most important factor in governing the time a fish takes to enter into, and pass through, *rigor mortis* since it can be controlled (Stroud, 1969). With coldwater species, lowering the temperature generally prolongs both the pre-rigor and the rigor periods (Amlacher, 1961). For example, in asphyxiated roach (*Leuciscus rutilus*) stored at 35°C, the pre-rigor period was only 30 min and rigor was resolved within 3 h of death. At 0°C, rigor was delayed for 24 h and it was 72-80 h in duration (Amlacher, 1961).

However, there have been isolated reports in the literature that some fish, particularly those from tropical waters, may experience a cold shock reaction when placed in ice (Giacelli, 1954; Nazir and Nagar, 1963; Pavar and Nagar, 1965; Disney et al., 1969; Sutcliffe, 1973). During iced storage of *Tilapia nilotica*, Disney et al., (1969) noted that the fish exhibited a rigor-like hardening which usually developed within 1 h. Uniced fish, however, did not enter *rigor mortis* until 2-5 h after capture.

Poulter, Curran and Disney (1981) studied this phenomenon further in stunned tilapia (*Oreochromis mossambicus*). They found that the onset of *rigor mortis* occurred after 2 h in ice (0°C) and 6 h at ambient temperature (22°C). Following this, a comparative study of the development of *rigor mortis* in tilapia (*Oreochromis aureus/niloticus* hybrid) and common carp (*Cyprinus carpio*) at the same temperatures was conducted (Curran *et al.*, 1984). The two species reacted quite differently to the two storage conditions. The tilapia left at ambient temperature started to stiffen 7 h after death and were in full *rigor mortis* after 19 h. The fish which were held at 0°C, however, exhibited the cold shock reaction and started to stiffen within minutes of being placed in the ice and full *rigor mortis* appeared to develop within 8 h. In contrast, the development of *rigor mortis* in carp was delayed until approximately 15 h after at both temperatures. The effect of temperature on the duration of *rigor mortis* was the same for both species in that lowering the temperature prolonged the *rigor* period.

3. POST-MORTEM METABOLISM OF FISH MUSCLE

The relationship between *rigor mortis* and post-mortem glycolysis and ATP degradation is highly complex (Eakin, Henderson and Townsend, 1971; Bendall, 1973; Lawrie, 1979) and has been the subject of a great deal of research with both mammals and fish. The death of an animal causes the blood circulation to cease and the muscles become anaerobic. The level of adenosine triphosphate (ATP) in the muscle eventually decreases, since oxidative phosphorylation will no longer occur, and this triggers the anaerobic conversion of glycogen to lactic acid, accompanied by a fall in pH.

Figures 1 and 2 indicate the changes which occur in ATP, lactic acid and pH during the development of *rigor mortis* and the cold shock reaction in tilapia and carp. These data indicate that temperature had different effects on post-mortem metabolism of the two species used in this study. In coldwater fish, post-mortem metabolism would be expected to occur at a slower rate as the temperature decreased (Tomlinson *et al.*, 1961; Fraser, Weinstein and Dyer, 1965; Portmann, 1965); the carp results for this and other studies (Noguchi and Yamamoto, 1955; Saito and Arai, 1957; Tomiyama *et al.*, 1966) are in agreement with this. The data for tilapia, however, indicate that their post-mortem metabolism did not appear to be greatly affected by the difference in temperature between iced and ambient storage conditions. In fact, the results from the preliminary work on tilapia (Poulter, Curran and Disney, 1981) appeared to indicate that the metabolism was stimulated at 0°C during the first 12 h post-mortem.

4. POST-MORTEM CONTRACTION OF MUSCLE FIBRES

Superficial comparisons have been drawn between the cold shock reaction in tropical fish and the phenomenon known as 'cold shortening' which occurs when certain mammalian muscles are chilled (Disney *et al.*, 1969; Poulter, Curran and Disney 1981). Lowering the temperature of pre-rigor bovine and ovine muscle from 37 to 5°C causes acceleration of the post-mortem metabolism which is accompanied by a severe contraction of the muscle fibres (Locker and Hagyard, 1963; Hamm, 1982). Cold shortening can be explained by the low temperatures inactivating the ATP-driven calcium pump and/or increasing the permeability of membranes of the sarcoplasmic reticulum or mitochondria to Ca^{2+} . This, together with appreciable levels of ATP, initiates muscular contraction before the onset of *rigor mortis*. The energy required for this shortening is supplied by an increased ATP turnover accompanied by accelerated glycolysis: myofibrillar ATPase is known to be stimulated by low temperatures in mammalian muscle (Cassens and Newbold, 1967) such that it is activated above 15°C and between 0 and 5°C.

If a drop in temperature from 37 to 5°C stimulates mammalian muscle to contract, rapid cooling of tropical fish from the temperature of their natural environment (25-30°C) to 0°C (in ice) might also cause stimulation of the muscle. An attempt was made to determine whether the cold shock reaction of tilapia was accompanied by shortening of the muscle fibres in the same way as cold-shortened mammalian muscle. Figure 3 shows the increased muscle tension in trout and tilapia held at 0 and 30°C. The results for both species agree with the data in the literature that the muscle contractions of *rigor mortis* were delayed by lowering the temperature, and the high temperature caused much more severe contractions (Amlacher, 1961; Burt *et al.*, 1970). In addition, the greater effect of lowering the temperature on the delay in contractions in tilapia compared with trout supports the theory that tropical fish are more greatly influenced by a drop in temperature than cold or temperate water fish (Poulter, Curran and Disney 1981). The result for tilapia indicates that the rapid extensive stiffening on chilling is not accompanied by muscle contraction, implying that, although the cold shock reaction in whole tropical fish is biochemically similar, it is not physically the same as cold shortening in mammalian muscles.

5. FILLETING YIELDS AND QUALITY OF TROPICAL FISH

Since *rigor mortis* alone can cause considerable problems for the fishing industry, the occurrence of a cold shock reaction in tropical fish could lead to additional complications during certain processing operations. Jones (1969) discussed the ways, other than its influence on

spoilage, in which *rigor mortis* affects fish; for example, water binding capacity; drip loss and processing yields; gaping or ragged appearance in fillets; suitability of fish for mechanical filleting; penetration of salt during curing; texture; colour, etc. Curran *et al.*, (1984a) investigated the effect of the cold shock reaction on some of these parameters. Tilapia (*Oreochromis mossambicus/niloticus* hybrid) fillets were prepared under three different handling procedures. The fish were either (i) filleted immediately after death (pre-rigor fillets); (ii) stored in ice for 3 days before filleting (post-rigor fillets) or (iii) 'aged' at ambient temperatures (22°C) for 6 h after death before icing and then filleted after 3 days (aged post-rigor fillets). All fillets were stored in ice for several days.

The filleting yields for the three handling treatments, pre-rigor, post-rigor and aged post-rigor, were 31.18, 27.79 and 30.62% respectively. They were all significantly different at the 5% level. The post-rigor filleting yield was also found to be significantly lower than those from the other two treatments at the 1% level. The pre-rigor fillets showed no drip loss but, instead, gained slightly in weight due to uptake of ice melt water even though they had been wrapped in waterproof paper during storage. Although there was more total free drip after six days on ice from the post-rigor fillets (1.4%) compared with the aged post-rigor fillets (1.1%), these levels were not found to be significantly different. The overall processing yields, calculated from the filleting yields and drip loss, were all significantly different with the greatest obtained from the pre-rigor handling treatment (31.40%) and the least from the post-rigor treatment (29.36%); the processing yield for aged post-harvest fillets was 30.29%.

The initial quality of all the fillets was found to be very good regardless of the handling treatment. The flesh of the pre-rigor fillets was white while that of both the post-rigor and aged post-rigor samples had a slight red coloration most of which disappeared during storage. Eight days after death the pre-rigor fillets started to develop off-odours and were considered to be spoilt the following day. At that time, the fillets from the two other treatments were still acceptable. Only approximately 20% of the pre-rigor fillets suffered from gaping whereas about 90% of both the post-rigor and aged post-rigor fillets were affected. For all three treatments, however, the gaping was a single longitudinal split along the fillet and would have only been awarded a score of 1 on the subjective scale of Love, Lavety and Steel (1969) which ranges from 0 (no gaping or longitudinal splitting) to 5 (fillet dropping to pieces).

6. DISCUSSION

The cold shock reaction of tropical fish has important commercial implications. The usual problems caused by *rigor mortis* in coldwater species, for which there is not always a clear solution, are accentuated by this phenomenon. The data which are currently available indicate that pre-rigor filleting of tropical species produces the best filleting and processing yields and the least drip loss and gaping. On-board filleting, which is often highly impracticable in the tropics, would be a requirement for a pre-rigor filleting operation. A further disadvantage is the shorter shelf life of the pre-rigor product. The FAO/WHO International Codes of Practice (FAO/WHO, 1977; FAO, 1982), which are widely accepted as being of considerable value, recommend that fish are chilled quickly to the temperature of melting ice soon after capture and are maintained at that temperature. However, icing tilapia immediately on capture, induces the cold shock reaction; low yields and more gaping are a consequence of filleting these fish after post-rigor.

Ageing tropical fish at ambient conditions for a few hours before icing could be the most relevant practice for tropical fish processing operations intending to produce fresh fillets. A delay in icing is usually the norm in tropical fisheries since it is often impracticable to take ice on board due to the limitations of space or the supply and distribution of the ice is such that it may not be readily available to, or is too expensive for, the fishermen. Several studies of delayed icing of tropical fish (Purwadi and Tempebolon, 1972; Disney, 1976; Jayaweera, *et al.*, 1980; Tikai, Olavides and Nugui, 1982; Wan *et al.*, 1982) have been carried out. These have often shown that a delay of up to 6 h prior to icing either has relatively little effect on the shelf life or still allows a sufficiently long storage period. It would be necessary to determine the most appropriate delay period (during which the fish would have to be protected from the drying effects of the sun and wind) for each operation.

Although more work is required in this area, in the light of evidence which is currently available, it would appear that the Codes of Practice will need to be revised to accommodate those procedures most suited to tropical conditions.

7. REFERENCES

- Amacher, E., *Rigor mortis* in fish. In *Fish as food*, edited by G. Borgstrom. New York, Academic Press, Vol. 1: 385-409.
- Bendall, J.R., Post-mortem changes in muscle. In *The structure and function of muscle*, Vol. 2 1973 Structure. Part 2, edited by G.R. Bourne. New York, Academic Press, pp. 243-309, 2nd ed.

- Bransnaas, F. and P. Hansen, Technological problems connected with *rigor mortis* in fish requiring more knowledge from fundamental research. In The Technology of fish utilization, edited by R. Kreuzer. London, Fishing News (Books) Ltd. for FAO, pp. 3-4
1965
- Burt, J.R., et al., Rigor tensions and gaping in cod muscle. J. Food Technol., 5:339-51
1970
- Cassens, R.G. and R.P. Newbold, Effect of temperature on the time course of *rigor mortis* in ox muscle. J. Food Sci., 32:269-72
1967
- Curran, C.A., et al., Cold shock reaction in iced tropical fish. J. Food Technol., (in preparation)
1984
- _____, et al., Effect of handling treatment on fillet yields and quality of tropical fish. J. Food Technol., (in preparation)
1984a
- Disney, J.G. The spoilage of fish in the tropics. In Proceedings of the First annual tropical and sub-tropical fisheries technological Conference. College Station, Texas A&M University, pp. 23-29
1976
- Disney, J.G., R.C. Cola, and N.R. Jones, Considerations in the use of tropical fish species. In Fishery products, edited by R. Kreuzer. Farnham, Surrey, Fishing News (Books) Ltd., for FAO pp. 329-37
1974
- Disney, J.G., et al., Quality assessment in *Tilapia* species. Rome, FAO, FE: FIC/69/0/29 (mimeo)
1969
- Eskin, N.A.M., H.M. Henderson, and R.J. Townsend, Biochemistry of foods. New York, Academic Press
1971
- FAO, Reference manual to codes of practice for fish and fishery products. FAO Fish.Circ., (750):
1982 257 p.
- FAO/WHO, Recommended international code of practice for fresh fish. Rome, FAO/WHO, CAC/RCP 9-1976
1977
- Fraser, D.I., H.M. Weinstein, and W.J. Dyer, Post-mortem glycolytic and associated changes in the muscle of trap- and trawl-caught cod. J. Fish. Res. Board Can., 22 (1): 83-100
1965
- Gianelli, F., Ricerche sul tempo di insorgenza e durata della rigidità cadaverica nella *Solea solea* in varia condizioni di ambiente e temperatura. Atti Soc. Ital. Sci. Nat., 8:464-5
1954
- Henn, R., Post-mortem changes in muscle with regard to processing of hot-boned beef. Food Technol.,
1982 36 (11):105-15
- Jayaweera, V., et al., Storage life of silverbelly (*Leiognathus* sp.) with delayed icing. Bull. Fish. Res. Stn., Sri Lanka, (30): 53-61
1980
- Jones, N.R., Fish preservation. Paper presented at the UNIDO Conference on Scientific approaches to the problems of preservation and refrigeration of food in developing countries, Vienna, 1969 (mimeo)
1969
- Lawrie, R.A., Meat science. Oxford, Pergamon Press, 3rd ed.
1979
- Locker, R.H. and C.J. Hayyard, A cold shortening effect in beef muscles. J. Sci. Food Agric., 14:
1963 787-93
- Love, R.M., J. Lavety, and P.J. Steel, The connective tissues of fish. 2. Gaping in commercial species of frozen fish in relation to *rigor mortis*. J. Food Technol., 4:39-44
1969
- Lovarn, J.A., Chemistry and advances in fish processing. Fish. News, 25:11
1952
- Nazir, D.J. and N.G. Magar, Biochemical changes in fish muscle during *rigor mortis*. J. Food. Sci.,
1963 28:1-7
- Noguchi, E. and J. Yamamoto, Studies on the 'arai' phenomenon (the muscle contraction caused by perfusing water), 4. On the *rigor mortis* of white meat fish and of red meat fish. Bull. Jap. Soc. Sci. Fish., 20:1023-6
1955

- Partmann, W., Changes in proteins, nucleotides and carbohydrates during *rigor mortis*. In The technology of fish utilization, edited by R. Kreuzer. London, Fishing News (Books) Ltd., for FAO pp. 4-13
1965
- Pawar, S.S. and N.G. Mager, Biochemical changes in catfish, tilapia and mrigal fish during *rigor mortis*. *J. Food Sci.*, 3:121-5
1965
- Poulter, R.G., C.A. Curran, and J.D. Disney, Chill storage of tropical and temperate water fish - differences and similarities. In *Advances in the refrigerated treatment of fish. Sci.Tech.Froid/Refrig.Sci.Technol.*, Paris, 111-23
1981
- Purwadi and Tampobolon, M., Icing of fish. 2. Icing of *Decapterus*, mackerel, small tuna and tawes. *Res.Rep.Inst.Fish.Technol., Jakarta*, (2):35 p.
1972
- Saito, T. and K. Arai, Studies on the organic phosphates in muscle of aquatic animals. 3. Effects of storage temperature upon the adenosine polyphosphate content of carp muscle. *Bull.Jap.Soc.Sci.Fish.*, 22:569-73
1957
- Stroud, G.D., Rigor in fish. The effect on quality. *Torry Advis.Note*, (36)
1969
- Sutcliffe, P.J., Report on visit to Ghana: experimental storage trials of iced fish. London, Tropical Development and Research Institute, Report (R351)
1973
- Tikai, T., M.G. Olavides, and C. Nugui, Effect of delayed icing on *Sphyrna* sp. Paper presented to the FAO/DANIDA Workshop on fish technology and quality control, 26 April - 4 June 1982, Manila, the Philippines
1982
- Tomiyama, T., et al., A study on the change in nucleotides and freshness of carp muscle during the chill storage. *Bull.Jap.Soc.Sci.Fish.*, 32:262-6
1966
- Tomlinson, W., Observations on post-mortem biochemical changes in fish muscle in relation to *rigor mortis*. *J.Fish.Res.Board Can.*, 18 (3):321-36
1961
- Tropical Products Institute, Background paper on the handling and processing of tropical fish and shellfish. Rome, FAO, FII:CF/75/2 (mimeo)
1975
- Wan, T.S., et al., The effect of icing and delayed on *Caranx leptolepis*. Paper presented to the FAO/DANIDA Workshop on fish technology and quality control, 26 April - 4 June 1982, Manila, the Philippines
1982

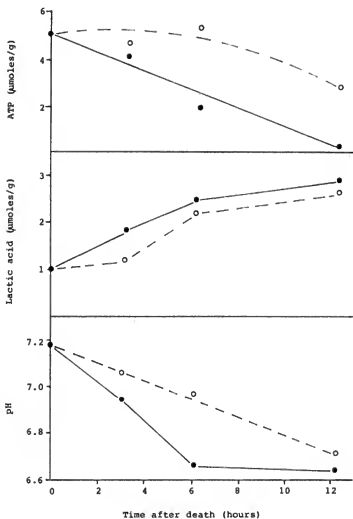


Figure 1 Changes in ATP, lactic acid and pH in tilapia (*Oreochromis mossambicus*) stored in ice (●—●) and at 22°C (○—○)
(Source: Poulter, Curran and Disney, 1981; Poulter and Mayall, unpublished data)

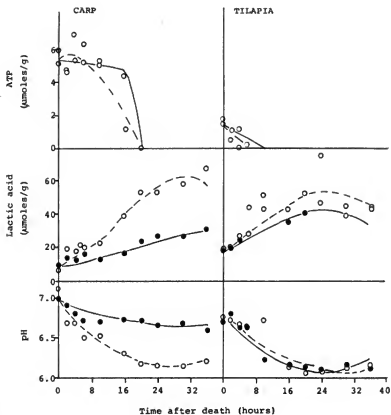


Figure 2 Changes in ATP, lactic acid and pH of carp (*Cyprinus carpio*) and tilapia (*Oreochromis aureus/niloticus* hybrid) stored in ice (—●—) and at 22°C (---○---). Regression lines are plotted.
(Source: Curran et al., 1984)

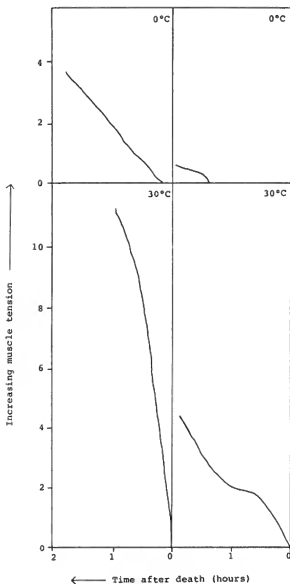


Figure 3 Development of tension (muscle contraction) in muscle fibres from trout and tilapia at 0 and 30°C
(Source: Curran *et al.*, 1984)

INTRODUCING NEW AND IMPROVED TECHNOLOGY USING QUALITY ASSURANCE PROGRAMMES

by

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ABSTRACT

Quality assurance programmes have proved useful in a number of situations in New Zealand. Uncomplicated pilot programmes were initially prepared as a guide to assist processors introduce quality assurance into their factory using existing staff and facilities. Individually tailored programmes have been most useful in introducing new products and technologies into industry particularly to those companies not having a high level of technical expertise. They have allowed development to take place by ensuring all those involved in the handling, processing and marketing of a product, have a clear picture of how and what is to be done.

1. INTRODUCTION

Quality Assurance Programmes where they have been adopted by some individual fishing companies have resulted in immediate improvements to existing product lines. These improvements have been achieved through the effective introduction of new technologies into their processing operations. The type and format of these programmes have been kept very simple so that existing factory employees rather than specialized quality control staff are able to operate the programmes. As well as maintaining control of processing, quality assurance programmes also have benefits in the marketing area. A written description of the product considerable assists buyers in understanding the exact nature of the product they are proposing to buy.

Draft quality assurance programmes for the fishing industry were first written in 1978 following a format programme for the meat industry (Oldfield, 1973) to meet a regulatory requirement that all fish processing factories introduce quality control systems. These were intended to act as a guide which the factories could follow by completing the appropriate sections with their own particular handling and processing requirements. These early programmes, because they had to serve as a guide to all processing factories regardless of size and method of processing, were somewhat generalized. For this reason and because the whole exercise was undertaken simply to comply with regulation the early programmes simply described the status quo and were of little benefit in improving overall quality. More recent programmes designed for particular companies wanting to:

- (a) improve product quality and had a definite quality goal in mind,
 - (b) wanted to introduce new products and/or new technology into their processing operations,
- have produced startling results.

Government and quasi Governmental agencies are very often given the responsibility of ensuring industry attains acceptable minimum quality standards. In practice, they also assume responsibility for assisting industry in improving overall quality, with industry tending to delegate their responsibilities for quality. The obligations of the agencies are discharged by:

- (a) legislative processes (factory construction and processing requirements, minimum hygiene and quality standards),
- (b) industry training programmes, seminars and technical advisory services,
- (c) codes of good commercial practice and information leaflets.

All these methods are useful in helping to achieve minimum quality standards and in ensuring that technical information does reach industry. However, in the authors' experience, improvements in existing technology or the adoption of new technology by industry via the above technology transfer methods, is a slow and gradual process. This is particularly so in industries where the numbers of highly trained technical people are low. It has also been observed that when new technology is

adopted the potential of that technology is seldom fully achieved due to inability to utilize the knowledge available. On the other hand it is also true to say that while fish scientists and technologists are striving to develop new and improved handling and processing methods there is a great deal of existing technology that is not yet fully utilized or only partially utilized. It is in the area of effectively introducing new technology to fishing companies that do not have a great deal of technical expertise that quality assurance programmes have been found to be particularly effective.

2. PROGRAMME CONSTRUCTION

Because virtually every factory uses a different processing method and different equipment, programmes are written specifically for each factory and for each product. The programmes themselves are also specific and avoid the use of generalities common with most codes of practice. Comments such as "frozen products should be transferred to the freezer store immediately after removal from the freezer..." mean very little to factory staff and are replaced with precise instructions on how product is to be transferred. For example, in that particular factory which used a tunnel freezer it was decided that to ensure product didn't warm up after freezing, staff would "transfer product to the freezer as soon as four cartons had accumulated". The programmes are kept as simple as possible, concentrating on the important information required to make the specific product in that specific factory. Information or requirements of a generalized nature are not included. Such things as factory construction requirements, hygiene facilities, etc., found in generalized codes of practice and other documents are assumed to have been met and the factory operates under good commercial practice. By doing this, one finishes up with a relatively small document that is in consequence easily read and understood. Further to this the programmes are divided up into five distinct sections that stand by themselves and can be read separately. This enables people to be given a concise summary of that part of the total processing operation which is of particular interest or concern to them.

The first and most important step in drawing up any programme is to decide exactly what product is to be made and to write down a clear description of the product. This relatively simple step is in most cases the hardest one to achieve. It involves bringing together the necessary market and technical information to allow an accurate and realistic description to be made. The contents of this description are summarized in Section 1 of Table 1. From experience it is clear that if the product description section has not been well thought through then preparing the other sections of the quality assurance programme becomes somewhat of an academic exercise. It is in this section that the objectives of any development programme are clearly defined. Vague concepts such as, "do it better", "do it cheaper", "we want the best", are of little value unless they are backed up with sufficient market and technical information to produce a clear picture as to "how it is to be done".

The process of preparing the product specification section is really only the formalisation of decisions made in preparing the product description. It specifies the quality of the product particularly as to its taste and appearance, unit weights, labelling and packaging used. Some of the more important handling criteria and storage quality aspects are also written into the specification. Chilling, freezing and storage temperatures and times in particular are specified to ensure that product at point of sale complies with the quality specification. The storage time is of critical importance and is determined by an evaluation of the storage conditions prevailing throughout the distribution chain. Because temperature control is not always ideal, maximum storage time before sale has to be set.

The raw material control and process specification sections can now be finalized. These are designed to ensure that the final product will consistently meet the product specification. Information to prepare these sections is most often available in codes of practice and other published literature. What is required here is a rewriting of that information to suit the conditions and equipment available in the factory for which the programme has been written.

The quality control procedures is the final section to be prepared. Because most of these programmes have been prepared for companies that do not have specialized quality control staff or laboratories, the procedures specified are mainly aimed at ensuring that the raw material control and process specification requirements are adhered to. The most effective checking procedures for controlling quality are those which the processing staff carry out themselves. Quality assurance programmes, because they provide a step by step outline of job function, help to educate staff in their work and increase the awareness of the quality of the product that they are producing. As a result of this increased knowledge the ability to maintain quality is considerably enhanced.

3. EXAMPLES

The first use of quality assurance programmes was in the development for export of two products made from farmed mussels, frozen half shell and live chilled mussels. The technology for both these products had been developed (Boyd and Wilson, 1978), but despite extensive seminars and other educational activities, expansion of the mussel industry was largely hindered by ineffective use of technology.

A quality assurance programme prepared for the half shell mussel product incorporated control of the harvesting operation as well as factory processing. It also included use of modern packaging techniques and limited the frozen storage period to ensure quality (Turner, 1984). Market acceptance of the product was such that other processors are now adopting similar methodology. It was not until all the various steps and activities were put together in one package that industry at large could visualize the process and the increased quality and marketability of the final product.

Live chilled mussels for export is another product that was assisted with quality assurance programming. Although the potential shelf life of correctly chilled mussels is at least 10 days, lack of temperature control throughout the distribution chain results in a product of low commercial acceptability if held for that period of time (Warwick, 1983). Quality assurance programming by one company has ensured the maintenance of temperature during distribution and the growth of their enterprise (Batley, 1984).

The export of high quality chilled southern bluefin tuna from New Zealand has been a fledgling industry for a number of years. The technology for handling bluefin is well known and an established industry internationally (Konagaya and Konagaya, 1979; Tanaka, *et al.*, 1974). The difficulty of preventing burning post-capture is a continuing problem but methods of containing this problem in the West Coast New Zealand fishery have been reported on (Wilson, 1982; Gibson, 1981). However, because many and varied methods have been suggested for the handling of bluefin tuna to prevent burning confusion has resulted in the industry as to the correct and most appropriate handling method. A quality assurance programme that incorporates the known technical data and which takes into account the physical conditions prevailing in the fishery allowed one company to decide exactly how it was to handle and process its fish. The programme was drawn up in such a way that both fishermen and factory staff had clear definitions as to their role and job.

Other programmes prepared include one for the manufacture of a smoked product using southern blue whiting and the processing of pasteurized crab meat (Wilson, pers. com.). The crab meat product was of particular interest because of its potential microbiological problem and because it needed process approval from the regulatory authority. Because this product had never previously been produced in New Zealand and because existing regulations did not adequately relate to this product, the ability to give approval to process became almost impossible. A quality assurance programme overcame this problem because it gave the authorities a clear picture of how the process worked and how it was to be controlled.

4. REFERENCES

- Batley, T., Private Communication. Pernaful Holdings Ltd., Blenheim, New Zealand
1984
- Boyd, N.S. and N.D.C. Wilson. Handling and processing of raftfarmed mussels. Proc. IPFC, 18(3):354-8.
1978
- Gibson, D.J.M., A handbook on processing southern bluefin tuna for the fresh chilled sashimi market
1981 in Japan. Wellington, New Zealand, Ministry of Agriculture and Fisheries.
- Konagaya, S. and T. Konagaya. Acid denaturation of myofibrillar protein as a main cause of formation
1979 of "yankeniku" a spontaneously done meat in red meat fish. Bull. Jap. Soc. Sci. Fish.,
45(2):245-6
- Oldfield, S., Quality Assurance programme for tripe. Report from Biotechnology Department, Massey
1973 University, New Zealand.
- Tanaka, T., *et al.*, Quality of tuna meat frozen aboard with reference to freshness of the fish and
1974 freezing conditions. In proceedings of the IIF/IIR Commission, B2 D3/Tokyo. Paris, -
IIF/IIR, pp. 287-95.
- Turner, J.A., Private Communication. Kivicams Ltd., Nelson, New Zealand.
1984
- Warwick, J.C., Handling, transport, distribution and storage of live green mussels. Report to
1983 New Zealand Fishing Industry Board.
- Wilson, N.D.C., Some aspects of the handling and processing of tuna in New Zealand's South Island
1982 west coast fishery. Rep. Food Technol. Res. Cent. Massey Univ. N.Z., (s)

Table 1

The construction of quality assurance programme

Section	Typical Contents	Use
1. Product Description	<ul style="list-style-type: none"> - What the product is - What it is made from - How it is packaged - Markings on the carton 	Information of interest to the buyer and regulatory authorities
2. Product Specification	<ul style="list-style-type: none"> - What the product looks and tastes like - Chilling, freezing, storage or other process requirements - Weight and/or count per carton - Other pertinent product performance data 	Describes what the processor is required to make and the buyer expects to receive
3. Raw material Control	<ul style="list-style-type: none"> - When and how fish is caught - How fish is to be handled at sea - Method of transport to factory 	Information required by fishermen or factory suppliers and factory staff. Provides minimum raw material standard
4. Process Specification	<p>Instructions for:</p> <ul style="list-style-type: none"> - Receiving raw materials - Storage prior to processing - Processing and packaging method - Product storage 	Working instructions for production management and staff
5. Quality Control Procedures	<p>List of checking procedures to ensure:</p> <ul style="list-style-type: none"> - Raw material meets requirements - Product processed according to instruction - Final product meets specification 	Identifies when process has gone wrong and indicates action to be taken

THE PROBLEMS OF BY-CATCH HANDLING AND ON-BOARD STORAGE OF FISH
LANDED AT SAMUT PRAKAN

by

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ABSTRACT

Approximately 800 000 t of by-catch is landed annually in Thailand. Most of this by-catch is of extremely low quality and is used mainly for fishmeal manufacture. More than half of the production is exported at a very low unit value compared to other exported products. Meanwhile, there is a population of over 10 million in the low income bracket requiring cheap proteinaceous foods. This study determined factors and problems affecting the quality of by-catch. The population in the study involved 499 trawler captains in Samut Prakan Province of Thailand. Fifty survey samples included in the study were selected by using a stratified random sampling method. An interview schedule was employed in collecting data and the major findings were that: (i) there was space, time and labour available for the improvement of the by-catch quality on board; (ii) that a better price for by-catch would be the main incentive to improve the quality for human consumption; and (iii) the most promising lot to be improved was the last day's catch.

1. INTRODUCTION

In Thailand, fish is the most important source of protein for human consumption as well as being a valuable export commodity. The population in 1983 was 49 515 074, with protein deficiency percent ranking highly as one of the national nutrition problems. In order to overcome this situation, there is an urgent need to reduce post-harvest losses and increase utilization of fish by-catch. Marine fishery resources could provide larger amounts of protein for human consumption if a systematic by-catch utilization and marketing system were developed. In 1981, the record of 796 747 t of by-catch landed was 43.7% of the total catch and comprised 80.6% of demersal species (Thailand, Department of Fisheries, 1983). Approximately 94.6% was used as raw material for fishmeal and the remainder utilized as animal feed or fertilizer (Thailand, Department of Fisheries, 1983). However, due to mishandling the industry ended up with a low quality, cheap raw material.

Thai fishmeal production was 186 201 t in 1982 (Thailand, Department of Fisheries, 1984) and 61.1% of this was exported with a value of only \$ 1 014 million (Thailand, Department of Fisheries, 1983). Exported seafoods amounted to 300 034 t valued at approximately \$ 8 775 million, of which fishmeal was 37.9% by weight, but only 11.6% by value. The total fish landed was reduced from 2 067 533 t in 1977 (Thailand, Marine Fisheries Department, Agricultural Economic Office, 1983) to 1 824 444 t in 1981 (Thailand, Department of Fisheries, 1983), but fish by-catch remained fairly constant.

These figures show that there was a smaller number of commercial species available over this period, but the population of Thailand continued to increase with an increasing demand for proteinaceous foods. By improving on-board handling, better quality raw material will be available to alleviate the protein deficiency problem of low-income groups. By using 10% of the by-catch for low-cost product development, 10 million people could be supplied with 8 kg per output.

This concept was the basis for our study on problems of by-catch handling and storage on board fishing vessels. Samut Prakan trawl fishery was chosen as being representative of the Thai trawl fisheries.

2. MATERIALS AND METHODS

There are 499 trawlers fishing out of Samut Prakan, 333 with other board trawls and 166 with pair trawls. Interview schedules which were presented were used to collect data from fishermen operating 50 trawlers using a stratified random sampling method.

Data was collected by five trained statisticians who interviewed captains of otter board trawlers in the Muang district and pair trawlers in the Klongdon area during June 1983. Data were processed and average figures were analysed. Trawlers were classified by horse power (hp) into three sizes: size 1 less than 200 hp, size 2, 200-300 hp and size 3 greater than 300 hp.

3. RESULTS AND DISCUSSIONS

The general characteristics of trawl fisheries are shown in Table 1. Trawlers remained at sea for 2-23 days and 1-3.6 days in port between fishing trips. When at sea, 1-19.5 days were spent fishing with 3-4.6 hauls per day and a total of 4.4-80.1 hauls per trip.

Otter board trawlers, sizes 1 and 2 remained at sea for 2 and 21.8 days, respectively, and pair trawlers sizes 1 and 2, 7.2 and 8.5 days, respectively.

The available free time, labour and storage space per trawler fishing trip is shown in Table 2. The estimated free time per day ranged from 12.27 h to 19.26 h. The otter board trawl free time was shorter than the pair trawl.

Larger otter board boats also had more free time than smaller boats ranging from 10.74 h to 15.11 h.

The estimated free labour time was more than 2-17 fishermen. The empty storage room was 1.2, 2.1 and 1.9 for otter board trawlers of sizes 1, 2 and 3, respectively, and 3.1 and 1.8 for pair trawlers of sizes 1 and 2. The volume of the storage space was 37.2 m³, 106.3 m³, 118.1 m³, 131.8 m³ and 91.1 m³, respectively.

The net benefit from improving the last day's by-catch is shown in Table 3. There was little difference in net benefit when plastic and zinc fish boxes were used to store fish, regardless of the sizes of trawlers.

Increase in costs in terms of labour time, ice and fish boxes are shown in Table 4, and the estimated increase in storage space for the last day of food grade by-catch is given in Table 5. The figures in Table 5 are estimates, based on the size of the ice boxes; however, the actual increase in storage will be higher when boxes are stacked. From the data in Table 2 sufficient free storage area is available on all boats to carry fish boxes.

4. CONCLUSION

Facilities on board trawlers are potentially available to improve the quality of by-catch, especially that from the last day of trawling. If fishermen obtain an incentive price of B 4.95 ± 0.76/kg, improvements will be carried out. (At the time when this survey was carried out the average price of food grade by-catch was B 2.24 ± 0.14/kg and B 2.22 ± 0.09/kg at Muang and Klongdon districts, respectively.)

5. REFERENCES

- Thailand, Department of Fisheries, Thai fishing vessels statistics, 1982. Bangkok, Thailand, 1983. Department of Fisheries
- _____. Statistics of Thai fisheries, 1981. Bangkok, Thailand, Department of Fisheries 1983
- _____. Statistics of fisheries factory, 1982. Bangkok, Thailand, Department of Fisheries 1984
- Thailand, Marine Fisheries Department, Agricultural Economic Office. Bangkok, Thailand, Marine Fisheries Department (mimeo) 1983
- Agricultural Economic Office, Marine fisheries situation, by-catch and fish meal. (Internal report) 1983

Table 1

Characteristics of trawl fisheries

Averages	Size of otter board trawl (HP)			Size of pair trawl (HP)	
	< 200	200-300	> 300	< 200	200-300
1. No. of days/ fishing trip	2	21.8	23.1	7.2	8.5
2. No. of days before/after fishing	1	3.3	3.6	1.0	1.0
3. No. of fishing days	1	16.5	19.5	6.2	7.5
4. No. of hauling/ day	4.4	4.4	4.1	3.0	3.0
5. No. of hauling/ trip	4.4	78.7	80.1	18.7	22.5

Table 2

Available free time, labour and space per trip of trawlers

Averages	Size of otter board trawl (HP)			Size of pair trawl (HP)	
	< 200	200-300	> 300	< 200	200-300
1. Estimated free time (h/day)	15.11	12.27	10.74 (hours.minutes)	19.11	19.26
2. Estimated free labour (persons/ day)	> 2.0	> 17.0	> 16.6	> 11.8	> 17.0
3. Fishermen on board	3.0	18.8	21.8	14.0	17.66
4. No. of empty storage rooms after fishing	1.2	2.1	1.9	3.1	1.8
5. No. of fish storage rooms	2.8	8.2	8.8	7.1	7.3
6. Estimated empty storage space (m ³)	37.2	106.3	188.1	131.8	91.1

- No. 1: From Annex Tables 1 and 2, No. 16
 No. 2: From Annex Tables 1 and 2, No. 10
 No. 3: From Annex Tables 1 and 2, No. 1
 No. 4: From Annex Table 3, No. 4
 No. 5: From Annex Table 3, No. 1
 No. 6: From Annex Table 3, No. 6

Table 3

Net benefit from the improved handling of by-catch
from the last day of fishing

Averages	Size of otter board trawl (HP)			Size of pair trawl (HP)	
	< 200	200-300	> 300	< 200	200-300
1. Estimated quantity of by-catch (kg)/day	133	1 859	1 812	1 473	1 640
2. Price (B)/kg of by-catch before improvement		2.24		2.22	
3. Income from last day by-catch before the improvement	297.92	4 164.16	4 058.88	3 270.06	3 640.80
4. Improvement in quantity (kg) of by-catch*	71	985	960	781	869
5. Incentive price (B) after improvement			4.95		
6. Income (B) from last day by-catch after improvement	351.45	4 875.75	4 752.00	3 865.95	4 301.55
7. Total quantity (kg) of last day sorted out by-catch	62	674	852	692	869
8. Income (B) from last day sorted out by-catch at the price before improvement	138.88	1 957.76	1 906.48	1 536.24	1 711.62
9. Total income (B) from the sorted out by-catch after the improvement	490.33	6 833.51	6 660.48	5 402.19	6 013.17
10. Total increase in income (B)	192.41	2 660.35	2 601.60	2 132.13	2 372.37
11. Total increase in expenditures (B)					
-using ice and plastic box	9.84	165.65	111.99	18.16	43.86
-using ice and zinc box	10.08	178.02	124.89	21.33	47.22
12. Net benefit (B)					
-using plastic box lot	182.57	2 503.70	2 489.61	2 113.97	2 328.51
-using zinc box lot	182.33	2 491.33	2 476.71	2 110.00	2 325.15

* Source: FTDD/IDRC Sorting and Yield Studies

No. 3: No. 1 x No. 2

No. 4: Refer to 53% sorting for food grade by-catch

No. 9: No. 6 + No. 8

No. 10: No. 9 - No. 3

No. 11: From Table 4, No. 8

No. 12: No. 10 - No. 11

Table 4

Estimated increase in the expenditure, labour and time consumed
for the improvement of last day by-catch

Averages	Size of otter board trawl (HP)			Size of pair trawl (HP)	
	< 200	200-300	> 300	< 200	200-300
1. Estimated quantity of by-catch (kg/day)	133	1,850	1,812	1,473	1,640
2. Estimated quantity (kg) of ice before improvement	0	109.44	300	339.57	289.67
3. Estimated value (B) of ice before improvement	0	29.00	79.5	89.99	76.76
4. Estimated quantity (kg) of ice after improvement	35.5	656.67	640	390.5	434.5
5. Estimated value (B) of ice after improvement	9.41	174.02	169.6	103.40	115.14
6. Increase in value (B) of ice after improvement	9.41	145.02	90.1	13.49	58.38
7. Estimated increase in cost (B) of containers					
- plastic boxes	0.43	20.63	21.89	4.07	5.48
- zinc boxes	0.67	33.00	34.79	7.84	8.84
8. Total increase in expenditure (B)					
- ice and plastic box	9.84	165.65	111.99	18.16	43.86
- ice and zinc box	10.08	178.02	124.80	21.33	47.22
9. Sorting time (h) in boats	3.10	43.36	42.28	34.36	38.27
10. No. of fishermen on board	3	18.8	21.8	14	17.66
11. Total increase in time (h) used by the available labour	1.03	2.31	1.93	2.45	2.19

No. 2: From Annex Table 6, No. 3

No. 3: Price of B 0.265/kg

No. 4: Reference No. 8

No. 5: Price of ice B 0.265/6 kg

No. 7: From Annex Table 7

No. 8: No. 7 + No. 6

No. 9: Rate of sorting 2.33 h/100 kg

No. 11: No. 9 + No. 10

Table 5

Estimated increase in storage space for the last day food grade by-catch

Averages	Size of otter board trawl			Size of pair trawl		
	< 200	200-300	> 300	< 200	200-300	> 300
1. Volume of 1 unit of fish storage room (m^3)	31.0	50.62	99.0	42.5	50.62	50.62
2. Space (m^3) needed to store containers of by-catch						
- plastic boxes	> 0.144	> 2.099	> 2.042	> 1.668	> 1.840	> 1.840
- zinc boxes	> 9.096	> 1.44	> 1.392	> 1.128	> 1.272	> 1.272
3. Ratio of space for containers to volume of fish storage room (1 unit)						
- plastic boxes	> 1/215.28	> 1/24.12	> 1/48.48	> 1/25.40	> 1/27.51	> 1/27.51
- zinc boxes	> 1/322.02	> 1/35.15	> 1/71.12	> 1/37.68	> 1/39.80	> 1/39.80

No. 1: From Annex Table 3, No. 5

No. 2: From volume of plastic box = $0.028 m^3/1$ boxNo. 3: From volume of zinc box = $0.024 m^3/1$ box

No. 2 + No. 1

Annex Table 1

Average time and labour used in one day of the otter trawl board trawl fishing boats for different activities

Factors involved	Size of boats (HP)		
	< 200	200-300	> 300
1. Fishermen on board	3.0	18.8	21.8
2. No. of hauling/day	4.4	4.4	4.1
3. No. of hours/day	3.0	4.04	4.13
4. Time (h) used for pre-hauling	0.40 (24 min.)	0.41 (25 min.)	0.63 (38 min.)
5. Labour (no. of persons) used for pre-hauling	2.0	5.0	7.0
6. Time (h) used for hauling/lifting	0.5 (30 min.)	0.41 (25 min.)	0.48 (29 min.)
7. Labour used for hauling/lifting	2.0	7.0	9.0
8. Time (h) used for activities no. 3, 4 and 6	3.90 (3 h, 54 min.)	5.02 (5 h, 1 min.)	5.24 (5 h, 14 min.)
9. Time (h) used for sorting, washing & storing	1.12 (1 h, 7 min.)	1.71 (1 h, 43 min.)	2.10 (2 h, 6 min.)
10. Labour (no. of persons) used for sorting, washing and storing	2.0	17.0	16.6
11. Time (h) for activities no. 4, 6 and 9	2.02 (2 h, 1 min.)	2.52 (2 h, 30 min.)	3.20 (3 h, 12 min.)
12. Free time (h) during each hauling	1.88 (1 h, 53 min.)	2.33 (2 h, 13 min.)	2.03 (2 h, 2 min.)
13. Free time (h) during hauling/day	8.27 (8 h, 16 min.)	10.16 (10 h, 10 min.)	8.38 (8 h, 23 min.)
14. Total mentioned time (h) [no. 8 x no. 2]	17.16 (17 h, 10 min.)	21.89 (21 h, 53 min.)	21.64 (21 h, 38 min.)
15. Not mentioned time (h)/day	6.84 (6 h, 50 min.)	2.11 (2 h, 7 min.)	2.36 (2 h, 22 min.)
16. Total estimated free time (h)/day	15.11 (15 h, 7 min.)	12.27 (12 h, 16 min.)	10.74 (10 h, 44 min.)

Annex Table 2

Average time and labour used in one day of the pair trawl fishing boats for different activities

Factors involved	Size of fishing boats (HP)	
	< 200	200-300
1. Fishermen on board	14.0	17.66
2. No. of hauling/day	3	3
3. No. of hours/haul	3.18 (3 h, 11 min.)	4 (4 h)
4. Time (h) used for pre-hauling	0.12 (7 min.)	0.08 (5 min.)
5. Labour (persons) used for pre-hauling	5	4.5
6. Time (h) used for hauling/lifting	0.51 (31 min.)	0.5 (30 min.)
7. Labour used for hauling/lifting	6	7.33
8. Time (h) used for activities no. 3, 4 and 6	3.81 (3 h, 49 min.)	4.58 (4 h, 35 min.)
9. Time (h) used for sorting, washing and storing	1 (1 h)	1 (1 h)
10. Labour (persons) used for sorting, washing and storing	11.8	17.0
11. Free time (h) during each hauling	1.63 (1 h, 38 min.)	1.58 (1 h, 35 min.)
12. Free time (h) during each hauling	2.18 (2 h, 11 min.)	3 (3 h)
13. Free time (h) from each hauling/day	6.54 (6 h, 32 min.)	9 (9 h)
14. Total mentioned time (h) [no.8 x no. 2]	11.43 (11 h, 26 min.)	13.74 (13 h, 44 min.)
15. Not mentioned time (h)/day	12.57 (12 h, 34 min.)	10.26 (10 h, 16 min.)
16. Total estimated free time/day	19.11 (19 h, 7 min.)	19.26 (19 h, 16 min.)

Annex Table 3

Average size of storage space available on board of the trawl fishing boats

Averages	Otter board trawl fishing boats - size (H.P.)			Pair trawl fishing boats size (H.P.)	
	< 200	200-300	> 300	< 200	200-300
1. Total fish storage room	2.8	8.2	8.8	7.1	7.3
2. Number of rooms used for ice storage ^a before fishing	2.4	7.1	7.7	4.2	5.5
3. Number of rooms used for fish storage after fishing	1.2	2.4	3.4	2.1	2.7
- Number of rooms used for economic fish					
- Number of rooms used for by-catch	0	3.8	3.5	1.9	2.8
4. Number of empty rooms after fishing	1.2	2.1	1.9	3.1	1.8
5. Spaces (m ³) of 1 empty room ^a	31.0	50.6	99.0	42.5	50.6
6. Total storage space (m ³)**	37.2	106.3	108.1	131.8	91.1

* From trawl fishing boat design survey in 1980 of the Marine Fisheries Division by Mr Thong Nadkratok

** No. 6: No. 4 x No. 5

Annex Table 4

Price of by-catch at Samut Prakan Province

Data	Price of by-catch (Bahts)	
	Muang District I (otter trawl boats)	Klong dan District (pair trawl)
5/6/83	2.00	2.00
10/6/83	2.14	2.17
15/6/83	2.17	2.17
20/6/83	2.14	2.14
25/6/83	2.60	-
30/6/83	2.14	-
5/7/83	2.14	2.14
10/7/83	2.16	2.30
15/7/83	2.35	2.30
20/7/83	2.20	2.20
25/7/83	2.20	-
30/7/83	2.20	2.20
5/8/83	2.20	2.20
10/8/83	2.25	2.30
15/8/83	2.35	2.35
20/8/83	2.50	-
25/8/83	2.35	2.35
30/8/83	2.20	2.20
Average	2.24 ± 0.14	2.22 ± 0.09

Annex Table 5
Some details on trawl fishery in Samut Prakan

Averages	Size of otter board trawl (HP)		Size of pair trawl (HP)	
	< 200	200-300	< 200	200-300
1. Number of fishing boats used for sampling	5	13	11	6
2. Boat length (m)	8.12	19.44	17.28	19.29
3. Weight of fishing boats (gross ton)	2.95	67.32	27.53	43.97
4. Fishing boat machine power (horse power)	128.00	265.00	174.09	229.16
5. Number of fishermen on board	3	18.8	14	17.66
6. Number of days/trip	2	21.75	7.2	8.50
7. Number of months of catching/year	12	12	12	12
8. Age (years) of fishing boat captains	35	40.88	35.68	38.50
9. Fishing experience (years) of captains	11	15.33	15.18	17.83

Annex Table 6

Ratio of ice to by-catch fish
practised on board otter board and pair trawl fishing boats

Averages	Size of otter board trawl fishing			Size of pair trawl	
	< 200	200-300	> 300	< 200	200-300
First period of fishing trip	0	1:2	1:3	1:1	1:1
Middle period of fishing trip	0	1.17:1	1:1.5	1.8:1	2:1
End period of fishing trip	0	9:1	3.17:1	2.27:1	3:1

Annex Table 7

Increase in cost of using fish containers for the improvement of last day by-catch

Averages	Size of otter board trawl			Size of pair trawl	
	< 200	200-300	> 300	< 200	200-300
Quantity (kg) of last day by-catch	133	1,859	1,812	1,473	1,640
Quantity of containers					
- plastic boxes	5	73	71	58	64
- zinc boxes	4	60	58	47	53
Cost (Baht) of boxes					
- plastic box/365 days	312.50	4,562.50	4,437.50	3,625.00	2,915.00
- zinc box/165 days	220.00	3,300.00	3,190.00	2,585.00	2,915.00
Increase in cost of boxes/day					
- plastic boxes	0.86	12.5	12.16	9.93	10.96
- zinc boxes	1.33	20.0	19.33	15.67	17.67
Number of day 8/1 using time	0.5	1.65	1.8	0.5	0.5
Cost/use in one trip					
- plastic boxes	0.43	20.63	21.89	4.97	5.48
- zinc boxes	0.67	33.0	34.79	7.84	8.84

PRELIMINARY STUDIES ON THE MICROBIAL CHARACTERISTICS
AND QUALITY OF VACUUM PACKED TRENCH SARDINES (*Amblygaster sirm*)
STORED UNDER REFRIGERATION CONDITIONS

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ABSTRACT

Trench sardines when dipped in potassium sorbate (2X) before vacuum packaging had a shelf life of 50 days at 4°C. By contrast, untreated or irradiated fish were rejected 26 and 22 days, respectively, after vacuum packaging and storage at 4°C. At 15°C shelf life of vacuum packed fish was 6-9 days irrespective of treatment (irradiated, sorbate treated, untreated). Interestingly, trench sardines dipped in saturated salt solution for 24 hours before vacuum packaging had a shelf life in excess of 50 days at 4°C. Unfortunately the consumption of saturated salted product was limited because of the unfavourable effect of high salting. Total bacterial counts at 30°C, initially 10⁴/g increased to 10⁶/g, at rejection with the exception of irradiated vacuum packed sardines with a total bacterial count of 10⁵/g at rejection. Although the initial microflora consisted of *Micrococcus*, *Bacillus*, *Pseudomonas* and *Enterobacteriaceae*, at rejection the predominant organism present was *Streptococcus*.

1. INTRODUCTION

Vacuum packing has recently been used as a means of improving the keeping quality of fresh fish at chill temperatures (Huss, 1971; Shewan and Hobbs, 1963; Murray and Shewan, 1969). Packaging protected fish from contamination and if vacuum packed, fat oxidation was inhibited. Hobbs and Hodgkiss (1982) stated that in vacuum packs the limited supply of oxygen or its complete absence inhibited the normal spoilage bacteria (*Pseudomonas* and *Alteromonas* sp.), leaving those bacteria that can grow well in anaerobic conditions to produce different metabolic products. However several studies claimed that either in aerobic packaging (Murray and Gibson, 1971) or in some instances vacuum packaging, only a marginal increase in shelf life was obtained (Huss, 1971; Gorczyca, 1983). Despite this Hobbs and Hodgkiss (1982) state that in addition to other commercial advantages, packaging could make fish more attractive and easier to handle.

Robach (1978) has shown irradiation (3 Cy or less) resulted in two to three fold extension in shelf life of many fishery products without perceptible alterations in the organoleptic properties. Similarly, chemical treatments, such as potassium sorbate, have been found to inhibit growth of most spoilers such as *Pseudomonas* sp. (Robach, 1978).

The present work examined the effect of pre-treatments, such as potassium sorbate, irradiation and salting on vacuum packed trench sardines stored at chill temperatures (0, 4 and 15°C).

2. MATERIALS AND METHODS

Trench sardines (*Amblygaster sirm*) were caught by gillnetting off the coast of Negambo, Sri Lanka. Fish samples were obtained from boats at the landing site around 8.00 a.m., washed lightly in tap water and transported in ice in an insulated box to the laboratory. On arrival the fish were beheaded, gutted and washed well in chilled water.

The fish were immersed in a supersaturated brine solution for 5-7 minutes, then gently shaken in fresh water for a few seconds and left to drain for a few minutes.

Potassium sorbate dip: after draining, dressed fish were dipped in a potassium sorbate solution (2X) for 15 minutes drained and then packed.

Irradiation: dressed fish were vacuum packed, transported in ice for irradiation, by a Co 60 irradiator. During irradiation at a dose of 2 Gy (200 Krad.), icing was continued.

Saturated salted fish: washed fish were immersed in supersaturated brine for approximately 24 hours followed by lightly washing and vacuum packaging.

Control samples (untreated): fish after draining, were packed in vacuum pouches.

2.1 Fish packaging and storage

Fish were packed in nylon/propylene laminated with oxygen permeabilities of 0.03 and 5 to $8 \text{ g}^{-1} \text{atm}^{-1} \text{ (20°C)}$ for the chill storage studies. Sealing was done using a "Bibun" (Bibun Company Japan) vacuum sealer.

All packs were stored at 15, 0 to -1°C (for trial 1) and 4°C (for trial 2). Microbiological, chemical and visual analysis was carried out at regular intervals.

2.2 Microbiological Analysis

Total bacterial counts (5, 15 and 30°C incubation temperatures) were estimated by plating ten fold serial dilutions of fish samples, prepared by homogenizing 50 g of fish meat, obtained through the body region, in 450 ml of sterile peptone (0.1%) diluent. Surface and pour plate techniques were employed in order to obtain total viable counts. Number of major representative colonies (obtained at 30°C/triplicate plating) were recorded, while 3-6 colonies of each type were restreaked on NA for purity and then stocked on NA slants stored at 4°C. Salted (saturated) samples were plated and stocked on seawater agar. Biochemical characterization of isolates was carried out according to the schemes of Shewen, Hobbs and Hodgkiss (1961), Cowan (1974) and Lee and Pfeifer (1975). All isolates were differentiated up to generic level.

In addition to aerobic counts, anaerobic counts were obtained from plates in anaerobic jars (BBL gas generating kits).

2.3 Chemical Analysis

Fish fillets (50 g) were minced to a smooth paste, of which 25 g was removed for (TVN) total volatile nitrogen and (TMA) trimethylamine (Anon., 1977). pH was measured for fish/distilled water macerates (also used for TVN) by using a Radiometer 26 pH.

2.4 Sensory Evaluation

Vacuum packs were examined visually for gas production (loss of vacuum) and appearance of fish (digestion) translucency, slime, sheen, liquefaction). After opening, the odour of packs was described as fresh, seaweedy, neutral hydrogen sulphide, ammoniacal, fruity and putrid. The texture of the fish was classified as firm or soft by 2-4 trained panelists.

3. RESULTS

At 4°C an extended shelf life of 50 days was obtained when trench sardines were dipped in potassium sorbate solution (2%), while the irradiated and untreated fish were acceptable up to 26 and 22 days, respectively. Saturated salt packs remained unspoiled even at 50 days of testing (deep frying or desalting of fish would be required prior to consumption, Table 1). By contrast, at 15°C, fish dipped in potassium sorbate kept for only 9 days, while untreated, irradiated and salted fish were acceptable for 6, 7 and 7 days, respectively (Table 1).

Sorbate dipped fish were found to be more or less softened due to the dip. However, rejection of sorbated fish was on strong ammoniacal odour at 4°C, which was preceded by a period of neutral odours. TVN increased from an initial value of 13.4 to 74.4 mg N/100 g fish at rejection (Table 2).

Irradiated packs stored at 4°C were rejected on the 26th day (Table 1) due to the development of fruity odours, with TVN at rejection being 40.8 mg (Table 2) and overall appearance remaining fairly shiny and firm.

Untreated samples were acceptable up to 22 days with packs giving off strong acidic odours at spoilage (Table 1). TVN values at rejection were 45.8 mg (Table 2).

At 15°C storage temperature, sorbate treated packs produced slightly sulphidic odours at rejection with retention of shiny appearance and slight bulging of pouches due to mild gas production (Table 1). TVN values at rejection were 60 mg (Table 3). Untreated samples were acceptable up to 6 days with the development of extensive (H₂S) gas (Table 1). Irradiated and salted packs were characterized by salty and seaweedy odours, with the latter pack showing signs of brown discolouration and the

former pack fermentation and digestion (Table 1). TVN of untreated packs at rejection were 68.9 mg and salted packs, 55.6 mg.

Packs stored at 0°C were rejected on the 27th day due to mild digestion, softening and putrid odours, with TVN values at 42.3 mg (Table 2).

Trimethylamine values showed no consistency and thus could not be used as an index of spoilage in vacuum packed trench sardines. pH ranged from 5.9-6.8 and not much variation was detected (Tables 2 and 3).

Total bacterial counts at 4°C storage ranged from <100 to 10^3 /g (5°C incubation) initially, whereas at rejection, counts of 10^5 - 10^6 /g (30°C, Table 1) were recorded. Irradiated packs at rejection, were at higher levels of 10^7 /g (15°C) shown in Table 3. Total anaerobic counts in all treatments were also similar to those obtained at 30°C. This finding indicated the facultative nature of the counts, for example, 9.6×10^5 /g at 30°C (aerobic) and 2.0×10^5 /g at 4°C (anaerobic) on sorbate treated samples (Table 2).

Composition of bacterial flora isolated from untreated fish packs at 4°C storage consisted of a mixture of *Enterobacteriaceae*, specifically *Enterobacter* sp. dominating and *Bacillus*. The flora on sorbate treated fish packs were dominated by *Pseudomonas* species, while those on vacuum packed fish with 2 Gy radiation were totally dominated (100%) by *Micrococcus*. In contrast, the flora on saturated salted packs were totally dominated (100%) by *Vibrio* sp. (Table 4).

The flora on spoiled: sorbate treated packs, control and irradiated pack was dominated by *Streptococci* (87-99%) as shown in Table 5. Saturated salt packs which were still acceptable had a flora consisting of *Streptococci* (24%) and *Micrococcus* (75%) on the 57th day.

Storing untreated and sorbate treated packs at 15°C however, caused a flora comprising of *Enterobacteriaceae*, mainly *Enterobacter* species dominating, were isolated initially. Also at 15°C storage, irradiated fish packs had a mixture of *Achromobacter*, *Enterobacteriaceae* and *Micrococcus*. Salt treated packs, like other treated packs have *Enterobacteriaceae* as the dominant flora, in addition to *Vibrio* species (Table 5).

Contrastingly, the flora while isolated initially were predominantly *Pseudomonas*, *Micrococcus* and *Bacillus* on packs stored at 0°C to -1°C. At rejection, the spoilage flora was predominantly *Pseudomonas* and *Micrococcus*. Consequently the absence of *Streptococci* at low temperatures (0 to -10°C) spoilage in contrast with the *Streptococci* domination (87-99%) previously mentioned was apparent.

4. DISCUSSION

Vacuum packaged trench sardines had a shelf life ranging from 20 days if untreated, to 50 days if sorbate dipped before packaging. This increase in shelf life after sorbate dipping may be due to the inhibition of microflora especially *Pseudomonas* sp. (Dainty, et al., 1979) commonly associated with fish spoilage.

Raw fish, although dominant in *Pseudomonas*, *Bacillus* and *Micrococcus*, once spoiled under vacuum, consisted of predominantly lactic acid bacteria, specifically *Streptococci*, for all treatments (Trials 1 and 2) except those stored at 0 to -1°C. *Streptococci* has previously been isolated from seafoods. Lee (1968) found *Leuconostoc* in Korean seafood pickles, whereas Orillo and Pederson (1968) found *Leuconostoc mesenteroides*, *Pediococcus cerevisiae* and *Lactobacillus plantarum* in Philippine fermented rice and fish (buro). Similarly Sands and Crisan (1974) observed *Pediococcus* present in fermented Korean seafoods. Huss (1971) reported reduced bacterial growth on vacuum packaged fish and a change in the spoilage association compared to aerobic controls. Nickerson, Goldblith and Proctor (1981) stated that packaging under low oxygen tension favoured selection and growth of *Lactobacilli* in irradiated seafoods which recorded an extended shelf life. McNeekin, Hulse and Bremner (1982) found that vacuum packed meat of normal pH, with an extended life supported the growth of *Lactobacilli* species.

In the present study, *Streptococci* may have contributed to the extended shelf lives in vacuum packed fish.

However inoculation studies by McNeekin, Hulse and Bremner (1982), Bremner and Stattham (1983) did not successfully induce the growth of added *Lactobacillus* or was spoilage flora inhibited.

Much shorter shelf lives (by 13 days) were obtained at 15°C compared with 4°C storage. At rejection, *Pseudomonas* (in untreated, salted and irradiated fish) and *Micrococcus* (in sorbate and salted fish) were the main microflora besides *Streptococci* at 15°C. The development of strong sulphid odours and gas production in the untreated packs stored at 15°C may be due to a specific *Pseudomonas*, *Alteromonas putrefaciens* which has been associated with spoilage in vacuum packed fish (Gorczyca, 1983).

Initial microflora on vacuum packed sardines was dominated by *Enterobacteriaceae*, predominantly, *Enterobacter* species. McMeekin, Hulse and Bremner (1982) also isolated *Enterobacter* sp. from vacuum packaged flathead filets at rejection. The filets were stored under reduced pH and added glucosa to enhance the growth of lactic acid bacteria, but only resulted in the reduction of *Alteromonas* spp. and an increase in *Enterobacter*. Further, *Enterobacter* have also been isolated from spoiling chicken held at 15°C (Bernes and Thornley, 1966) and spoiling fish stored at 28°C (Gorczyca and Pek Pohn Len, these proceedings).

Low dose (0.07 to 0.23 Gy) irradiation generally eliminated *Pseudomonas* species without affecting *Achromobacter* and Gram positive organisms, for example, *Microrosoci* (Liston, 1980). In the present study, initial microflora, after irradiation (2 Gy) of vacuum packs, consisted of *Microrosoci*, irrespective of the storage temperature, 4 or 15°C. *Achromobacter*, by contrast, were only isolated from packs stored at 15°C.

Interestingly, Palroy and Eklund (1966) recorded the growth of *Pseudomonada* in fish packs stored at 4°C, even though the packs had been irradiated (2 Gy). Thus, the fruity odours, recorded at rejection of irradiated packs (stored at 4°C), may be due to presence of *Pseudomonada*, specifically, *Pseudomonas fragi* (Bernes and Thornley, 1966). Liston (1980) postulated that since irradiated fish had a selected bacterial population, the numbers of bacteria necessary for spoilage to develop would be higher in an irradiated pack than in an untreated pack. Such an observation was recorded in the present study, when the total bacterial count of the irradiated pack reach 10^6 /g at rejection, 1 log scale higher than for the untreated fish.

Extended shelf lives (50 days) for sorbate treated fish in vacuum packs (stored at 4°C) may be due to the effectiveness of sorbate to inhibit a wide range of organisms including: *Pseudomonas* spp., *Pseudomonas fragi* and *Alteromonas putrefaciens* (Moustafa and Collins, 1968; Robach, 1978; Beuchet, 1980; Chung and Lee, 1981 and Gorczyca, 1983). Further, Gorczyca (1983) found that hydrogen sulphide (H_2S) producing bacteria, *Alteromonas putrefaciens* a suspected spoiler of chilled fish, was inhibited throughout the storage trial, that is, more than 43 days by potassium sorbate. Chung and Lee (1981) suggested that the inhibition of spoilers may account for the lag in bacterial growth and therefore be responsible for the observed extension in shelf life of sorbate treated fish. In the present study, the lack of strong sulphidic "off-odours" in sorbate treated sardines, further supports Gorczyca's (1983) observation.

Potassium sorbate has also been found to effectively inhibit pathogens such as *Vibrio parahaemolyticus*, *Staphylococcus aureus* (Robach and Stetler, 1980) and *Clostridium perfringens* as well as *Clostridium botulinum*. Many workers (Tompkin, 1974; Beuchet, 1980; Smoot and Pierson, 1981 and Huhtanen, et al., 1981) have found potassium sorbate to have antibotulinal effects.

It has been feared that on the reduction of the normal spoilage flora by sorbate treatment or irradiation the associated shelf-life extension would provide sufficient time for *Clostridium botulinum* to grow. However, it was observed that abnormal and defective cell grow at pH 7.0-7.2, when potassium sorbate concentration was 1 to 2%. Similarly, Beuchet (1980) showed positive effects at sorbate concentrations of 0.003 µg/ml at pH 6.7. Further, Abrahamson, Silva and Molin (1965) indicated that vacuum packaging only showed a slight increase in the rate of toxin production when compared with aerobic packs.

Salted samples seem to have an extended shelf life but consumption would be limited because of the high salt content. Spoilage in salted packs seem to be associated with moderate halophilic (isolated on NA with 8% NaCl) *Pseudomonas* and *Microrosoci* strains.

Trimethylamine was found to be an unsuitable index of quality because no sequence with time was observed in the present study. This may be due to lack of precursors TMAO (Dyer and Wood, 1947) or the reduced numbers of bacteria capable of producing TMAO (Laycock and Regier, 1971). By contrast, TVN values showed steady increases during storage with values ranging from 50 mg initially to 60 mg N/100 g flash at rejection as when compared with visual observations.

In conclusion, a suitable shelf life of 50 days, as obtained after sorbate dipping and vacuum packaging, give trench sardine packs a marketable shelf life and extensive distribution. However, strict temperature control, below 3.5°C, during marketing and distribution is recommended to prevent growth of *Clostridium botulinum* Type E. As storage temperature increases, not only is the shelf decreased but the risk of *Clostridium botulinum* growth increased.

5. REFERENCES

- Abrahamson, K., N.N. de Silva and M. Molin, Toxin production by *Clostridium botulinum* type E in vacuum packed, irradiated fresh fish in relation to changes of the associated microflora. *Gen.J.Microbiol.*, 11:523-9
- Bernes, E.M. and M.J. Thornley, The spoilage flora of eviscerated chickens stored at different temperatures. *J.Food Technol.*, 1:113-9

- Beuchat, L.R., Comparison of anti-*Vibrio* activities of potassium sorbate, sodium benzoate and glycerol and sucrose esters of fatty acids. Appl.Environ.Microbiol., 39:1178-82
- Bremner, H.A. and J.A. Statham, Effect of potassium sorbate on refrigerated storage of vacuum-packed scallops. J.Food Sci., 48:1042-7
- Chung, Y.M. and J.S. Lee, Inhibition of microbial growth in English sole (*Parophrys retulus*). 1981 J.Food Prot., 44:66-8
- Cowan, S.T., Cowan and Steel's manual for identification of medical bacteria. Cambridge, Cambridge University Press, 238 p.
- Dainty, R.H., et al., The spoilage of vacuum-packed beef by cold tolerant bacteria. Tech.Ser.Soc. Appl.Bacteriol., 13:88-100
- Dyer, F.E. and A.J. Wood, Action of *Enterobacteriaceae* on choline and related compounds. J.Fish. Res.Board Can., 7(1):17-21
- Gorczyca, E.M., Studies of the shelf life extensions of retail fish fillets. M.Appl.Sci.Thesis, 1983 Applied Chemistry Department, RMIT, Australia
- Hobbs, G. and W. Hodgkiss, The bacteriology of fish handling and processing. In Development in food microbiology. 1, edited by R. Davies. London, Applied Science Publishers, pp. 71-80
- Huhtanen, C.H., et al., Flavour and anti-botulinal evaluation of sorbic acid-containing bacon. 1981 J.Food Sci., 46:1796-801
- Huss, H.H., Prepackaged fresh fish. In Fish inspection and quality control, edited by R. Kreuzer, 1971 London, Fishing News (Books) Ltd., for FAO, pp. 60-5
- Laycock, R.A. and L.W. Regier, Trimethylamine-producing bacteria on haddock (*Melanogrammus aeglefinus*) fillets during refrigerated storage. J.Fish.Res.Board Can., 28(3):305-9
- Lee, J.S. and D.K. Pfeifer, Microflora associated with Dungeness crab (*Cancer magister*). Appl. Microbiol., 30:70-3
- Lee, K.M., Microbiological and enzymological studies on the flavour components of seafood pickles. 1968 J.Korean Agric.Chem.Soc., 11:1
- Liston, J., Microbiology in fishery science. In Advances in fish science and technology, edited 1980 by J.J. Connell. Farnham, Surrey, England, Fishing News (Books) Ltd., pp. 138-58
- McMeekin, T.A., L. Hulse and H.A. Brenner, Spoilage association of vacuum packaged sand flathead (*Platycephalus bassensis*) fillets. Food Technol., Aust., 34:278-82
- Moustafa, H.A. and E.B. Collins, Effects of selected food additives on the growth of *Pseudomonas fragi*. J.Dairy Sci., 52:335
- Murray, C. and D. Gibson, Prepacked chilled fish production. Torry Advis.Note, (52) 1971
- Murray, C.K. and J.M. Shevan, Preparation, handling and raiting of prepackaged wet fish. 1969 Packag.Technol., 5:20-5
- Nickerson, J., S.A. Goldblith and B.F. Proctor, A comparison of chemical changes in mackerel tissues treated by ionizing radiation. Food Technol., 4:84-8
- Orillo, C.A. and C.S. Pederson, Lactic acid fermentation of burong dalag. Appl.Microbiol., 16(11):1669-71
- Pelroy, G.A. and H.W. Eklund, Changes in the microflora of vacuum-packed irradiated Petrale sole (*Scopaeetus jordani*) at 0.5°C. Appl.Microbiol., 4:921-7
- Robach, M.C., Effect of potassium sorbate on the growth of *Pseudomonas fluorescens*. J.Food Sci., 1978 43:1886-7
- Robach, M.C. and C.L. Stateler, Inhibition of *Staphylococcus aureus* by potassium sorbate in combination with sodium chloride, tertiary butylhydroquinone, butylated hydroxyanisole or ethylenediamine tetracetic acid. J.Food Prot., 43:208-11

- Sands, A. and E. Crisan, Microflora of fermented Korean seafoods. J.Food Sci., 39:1002-5
1974
- Shewan, J.M. and G. Hobbs, Prepackaging unfrozen fish. Fish.News Int., 2:103-5
1963
- Shewan, J.M., G. Hobbs and W. Hodgkiss, A determinative scheme for the identification of certain
1961 genera of bacteria with special reference to *Pseudomonaceae*. J.Appl.Bacteriol.,
23:463-8
- Smoot, L.S. and H.D. Piersen, Mechanisms of sorbate inhibition of *Bacillus cereus* (T) and
1981 *Clostridium botulinum* (62A) spore germination. Appl.Environ.Microbiol., 42:477-83
- Tompkin, R.B., Effect of potassium sorbate on *Salmonella staphylococcus aureus*, *Clostridium*
1974 *perfringens* and *Clostridium botulinum* in cooked uncured sausage. Appl.Microbiol.,
28:262-4
- Anon., A collection of analytical methods and testing procedures for the assessment of fish and
1977 shellfish quality. Paper presented at the CIDA/FAO/CECAF training course
(TF/INT/180(f)(CAN)) on fish handling, plant simulation, quality control and fish
inspection, Dakar, Senegal, 10 October - 4 November 1977

Table 1

Shelf life of vacuum packed *Amblygaster sirm*

Treatment	Storage temp. (°C)	Shelf life ^{a/} (days)	Criteria for rejection	Other observation
Untreated	15	6	extensive gas, H ₂ S odour	no digestion/elim
Potassium sorbate dip (2%)	15	9	little gas, mild H ₂ S odour	no digestion/elim
Irradiated (2Gy)	15	7	brownish slime/acidic odour	no gas
Saltad (5% NaCl)	15	7	fermented odour	no gas
Untreated	4	22	acidic/slight digestion	no gas
Potassium Sorbate dip (2%)	4	50	NH ₃ odour/softening	no gas
Irradiated (2Gy)	4	26	fruity odour	shiny/no digestion
Salted (5% NaCl)	4	>57	-	-
Untreated	0	42	putrid odour	slight digestion

^{a/} Shelf life was the last day the trench sardines were acceptable to the taste panel

Table 2

Change in TVN, TMA, pH and bacterial count in vacuum packed *Amblygaster sirm* stored at 4°C

Analysis	Change in chemical and microbiological parameters							
	Untreated		Potassium sorbate dip		Irradiation		Saltad	
	0 ^{a/b/c/}	Rej. ^{a/b/c/}	0	Rej.	0	Rej.	0	Rej.
TVN ^{a/}	17.6	45.8	13.4	17.4	13.1	52.7	20.3	35.5
TMA ^{a/}	8.2	5.1	2.8	6.2	3.5	7.6	7.0	-
pH	6.8	6.2	6.3	6.4	6.0	6.7	6.2	6.0
TBC (5°C)	<100	3.7x10 ⁵	<100	1.0x10 ⁶	-	1.5x10 ⁷	<200	10x10 ²
TBC (30°C) ^{a/b/}	2.9x10 ³	2.0x10 ⁶	1.2x10 ³	9.6x10 ⁵	6.9x10 ³	6.0x10 ⁶	7.6x10 ³	9.0x10 ²
TBC (35°C) An ^{a/b/}	2.0x10 ²	6.2x10 ⁵	<100	2.0x10 ⁵	3.5x10 ²	7.3x10 ⁷	2.6x10 ³	1.0x10 ²

^{a/} Total volatile nitrogen (TVN), Trimethylamine (TMA) in mg N/100 g flesh^{b/} TBC - total bacterial count number/g^{c/} Rejection (Rej.) and Initial (0)

Table 3

Changes in TVN, TMA, pH and bacterial count in vacuum packed *Amblygaster sirum* stored at 15°C

Analysis	Changes in chemical and microbiological parameters							
	Untreated		Potassium sorbate dip		Irradiation		Salted	
	0 ^{a/b/c/}	Rej. ^{a/b/c/}	0	Rej.	0	Rej.	0	Rej.
TVN ^{a/}	18.8	56.4	22.0	60.0	44.4	68.9	21.1	55.6
TMA ^{a/}	10.4	9.8	5.6	7.1	11.3	11.3	10.3	9.1
pH	5.9	6.2	5.9	5.9	6.2	5.9	6.0	6.1
TBC (5°C) ^{a/b/}	8.6x10 ⁶	6.4x10 ⁵	7.2x10 ³	3.0x10 ⁸	1.1x10 ⁵	4.3x10 ⁶	2.8x10 ⁴	2.9x10 ⁶
TBC (30°C) ^{a/b/}	1.1x10 ³	1.7x10 ⁵	6.0x10 ²	3.2x10 ⁸	1.7x10 ⁵	1.4x10 ⁷	1.7x10 ⁵	3.2x10 ⁶
TBC (35°C) ^{a/b/} _{AN}	4.7x10 ³	1.3x10 ⁶	1.5x10 ³	5.9x10 ⁸	---	---	---	---

a/ Total volatile nitrogen (TVN), Trimethylamine (TMA) in mg N/100 g flesh

b/ TBC - total bacterial count number/g

c/ Rejection (Rej.) and Initial (0)

Table 4

Dominant types of microflora^{a/} isolated from vacuum packed *Amblygaster sirum* stored at 4°C and 0°C

Treatments	Microflora distribution (%)		Microflora distribution (%)	
	6 days		29 days	
Untreated	^{a/b/} <u>antarobacteriacae</u>	67.0	<u>straptococci</u>	99.5
	<u>Bacillus</u>		<u>psaedomonas</u>	0.5
	<u>psaedomonas</u>	33.0		
Potassium sorbate	<u>psaedomonas</u>	89.4	<u>straptococci</u>	96.9
	<u>Bacillus</u>	9.7	<u>staphylococcus</u>	
	<u>enterobacteriacae</u>	1.0	<u>psaedomonas</u>	4.0
Irradiation	<u>Micrococci</u>	100	<u>straptococci</u>	87.8
			<u>psaedomonas</u>	12.2
Salted	<u>Vibronaceae</u>	100	<u>vibronaceae</u>	
			<u>micrococci</u>	76.0
			<u>streptococci</u>	24.0
			<u>micrococci</u>	100
			<u>psaedomonas</u>	
Untreated (0°C)	<u>psaedomonas</u>	---	<u>psaedomonas</u>	---
	<u>micrococci</u>	---	<u>Bacillus</u>	---
			<u>micrococci</u>	---

a/ Obtained by triplicate sampling and averaged major representative colonies on NA plates incubated at 30°C

b/ antarobacter isolated

c/ on 8% salt/NA

Table 5

Dominant types of microflora^{a/} isolated from vacuum packed
Amblygaster sirm stored at 15°C

Treatment	Microflora distribution (%)	Microflora distribution (%)
	<u>1 day</u>	<u>7-9 days</u>
Untreated	<u>a/b/</u> enterobacteriaceae 74.0 Flavobacterie/cytophage 26.0	streptococci } 99.6 <u>a/b/</u> pseudomonas } enterobacteriaceae 0.4
Potassium sorbate	<u>a/b/</u> enterobacteriaceae 100	<u>micrococcus</u> } 89.3 streptococci } <u>pseudomonas</u> 3.5
Irredieted	<u>Achromobacter</u> } enterobacteriaceae } 66.3 micrococci 29.2 <u>pseudomonas</u> 4.5	bacillus 11.9 streptococci 85.8 <u>pseudomonas</u> micrococci 2.9 <u>pseudomonas</u> 0.4
Salted	<u>Vibrionaceae</u> } enterobacteriaceae } 100	<u>pseudomonas</u> } micrococci } 100

a/ Obtained by triplicate sampling and averaged major representative colonies on NA plates incubated at 30°C

b/ enterobacter isolated

c/ on 8% salt/NA

THE EFFECT OF FREEZING AND FROZEN STORAGE
ON THE QUALITY OF CHUB MACKEREL (*R. KANAGURTA*)

by

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ABSTRACT

Chub mackerel (*Rastrelliger kanagurta*) were frozen and stored at -20°C for four months. Two freezing rates were employed, i.e., fast (blast freezing) and slow (still-air freezing) using gutted fish. The fish were stored glazed and unglazed. Results indicated that the thiobarbituric acid (TBA) values increased with frozen storage, especially in the unglazed samples. Freezing rate did not affect TBA values except in the glazed samples. The solubility of the muscle proteins in an aqueous solution of 5% NaCl decreased with storage. pH values also decreased. Generally, quality deterioration in terms of fat oxidation and protein denaturation was more pronounced in unglazed samples.

1. INTRODUCTION

Although the market for frozen fish is limited in Malaysia at present, increasing urbanisation is bringing about greater utilization of frozen food products. There is also an export market for good quality frozen fish.

In this study, objective measurements were used to determine the keeping quality of fast- and slow-frozen chub mackerel during storage at -20°C .

2. MATERIALS AND METHODS

Chub mackerel (120 g) caught from the South China Sea were gutted and divided into four groups. Two groups were used for glazed storage and the other two for unglazed storage. For fast freezing, a blast freezer at -20°C was used. For slow freezing, a still-air freezer at the same temperature was used. After 24 h, all the samples were transferred into the still-air freezer for storage at -20°C . At predetermined intervals, samples from each group were thawed, filleted and ground for analysis.

Soluble protein nitrogen (Ironside and Love, 1958), thiobarbituric acid (Vyncka, 1973) and pH values were measured.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The proximate composition of chub mackerel is shown in Table 1. Similar results have been obtained for *R. brachyoma* (Foulter, Nicolaidis and Hector, 1978).

3.2 Fat Rancidity

For unglazed samples at 5% and 0.1% probability, t-tests showed no significant differences between fast- and slowly frozen samples in the rate and quantity of malonaldehyde produced (Figure 1). For glazed samples at 5% probability level, there was a slight significant difference in malonaldehyde values.

For both freezing rates, the differences in malonaldehyde values between glazed and unglazed samples were slightly significant at 5% and 0.1% probability level. Unglazed samples deteriorate more rapidly during storage.

3.3 Soluble Protein Nitrogen

Figure 2 shows the percent soluble protein nitrogen of chub mackerel in 5% NaCl solution for up to four months storage at -20°C . As usual with SPM determinations, the scatter is high (Connell,

1968). There are no detectable differences in all samples and no large decrease with storage. Similar observations have been reported with *R. brachyotoma* from Brunei (Poulter, Nicolaides and Hector, 1978). Poulter and Lawrie (1977) reported a 50% decrease in percentage SPN for *Clupea harengus*, a cold-water pelagic fish. It has been established that spoilage of tropical fish is slower than that of cold-water fish (Disney, Cole and Jones, 1974).

3.4 pH Changes

For all samples examined, pH changes were slight (Figure 3). In view of the little changes in pH and SPN values, it would be expected that the texture of the fish would have remained fairly constant throughout the storage period (Cowie and Little, 1966).

Table 1

Proximate composition of *R. kanagurta*

% w/w	Mean	Range	No. of replicates
Crude protein	22.03	24.57 - 20.79	7
Total lipid	2.08	2.71 - 1.48	8
Moisture	73.40	76.26 - 72.81	7
Ash	2.49	2.95 - 2.03	7

4. REFERENCES

- Connall, J.J., Changes in amount of myosin extractable from cod flesh during storage at -14°. 1968 J.Sci.Food Agric., 13:607-17
- Cowie, W.P. and W.T. Little, The relationship between the toughness of cod stored at -29°C and muscle protein solubility and pH. J.Food Technol., 1:335-43
- Disney, J.G., R.C. Cole and N.R. Jones, Considerations in the use of tropical fish species. In Fishery products, edited by R. Kreuzer. Parnham, Surrey, England, Fishing News (Books) Ltd., for FAO, pp. 329-37
- Ironside, J.I.M. and R.M. Lova, Studies on protein denaturation in frozen fish. Pte 1-3. J.Sci. Food Agric., 9:597-617
- Poulter, R.G. and R.A. Lawrie, Studies on fish muscle protein. Nutritional consequences of the changes occurring during frozen storage. J.Sci.Food Agric., 28:701-9
- Poulter, R.G., L. Nicolaides and D.A. Hector, Quality changes in fish from the South China Sea: iced storage of chub mackerel, grouper and fusilier. Proc.IPFCC, 18(3):169-85
- Vyncke, W., Evaluation of direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scomber* L.). Paper presented at the meeting of the Joint FAO/WHO Expert Committee on Fish and Shellfish Hygiene, Geneva

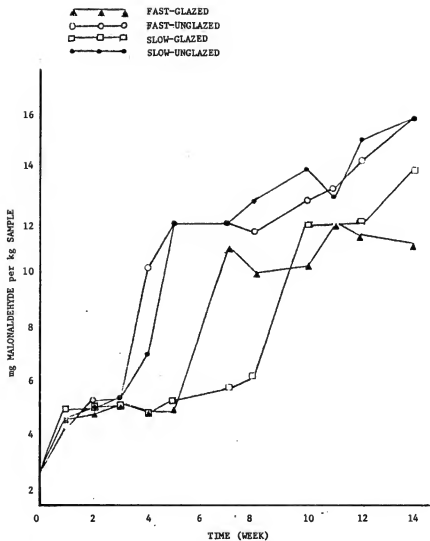


Figure 1 Malonaldehyde values of chub mackerel (*Rastrelliger kanagurta*) during frozen storage at -20°C

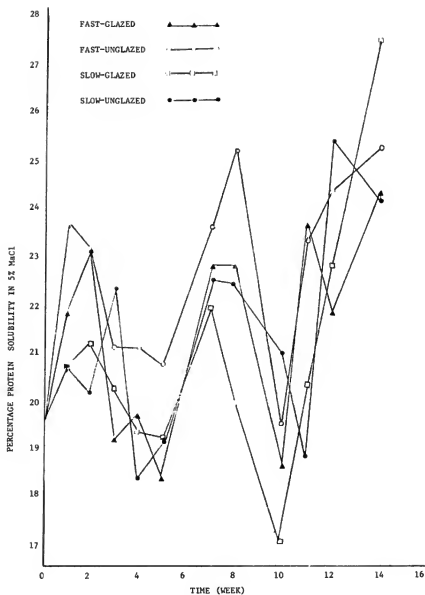


Figure 2 Protein solubility of *Rastrelliger kanagurta* during storage at -20°C

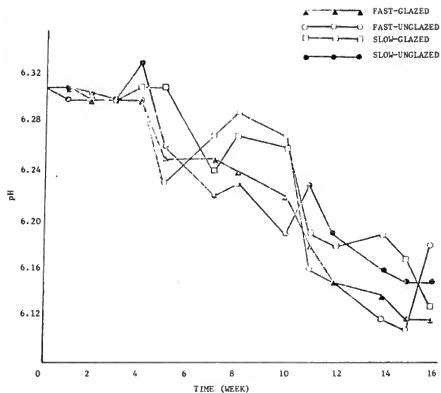


Figure 3 pH changes in chub mackerel (*Rastrelliger kanagurta*) during frozen storage at -20°C

INTRODUCTION TO THE HAZARD ANALYSIS CRITICAL CONTROL POINT
(HACCP) CONCEPT FOR CANNED FISH MANUFACTURE

by

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ABSTRACT

In response to the need for canned fish exporters to meet the stringent import regulations of the United States and European countries, the Food and Agriculture Organization has sponsored a training course on the principles and application of the HACCP concept for canned fish manufacture. The objective of the programme is to provide national institutes a formal procedure for evaluating in-process control techniques and the adequacy of thermal processing schedules, so that manufacturers (and importers) will no longer need to rely on limited terminal analysis to assure the safety of their products. Training commenced in Thailand in August at the Fishery Technological Development Division of the Ministry of Agriculture and Cooperatives. Lectures and pilot-plant exercises familiarised participants with techniques for identifying critical control points, after which operations in two canneries were reviewed. It is planned to monitor production across the entire canned export industry and provide each manufacturer with a confidential report assessing compliance with the United States Food Manufacturing Practice Regulations for low-acid canned foods. Other countries of the Indo-Pacific region to be included in the training programme are Indonesia, Malaysia and the Philippines where courses will commence in October and November 1984 and February 1985, respectively.

1. INTRODUCTION

Canned fish manufacturers supplying the American market are required by the United States Food and Drug Administration to comply with strict import regulations in relation to registration, process filing and manufacture according to Good Manufacturing Practice (GMP) guidelines. In order to assist canned fish exporters from the IPFC region, FAO has sponsored a training course on the principles and application of the Hazard Analysis Critical Control Point (HACCP) concept for canned fish manufacture. The programme is to provide the national institutes of Thailand, Indonesia, Malaysia and the Philippines and the regulatory authorities from these countries, a formal procedure for evaluating in-process control techniques and the adequacy of thermal process schedules. Once implemented, the scheme will remove the need for manufacturers (and importers) to rely on limited and often unrealistic terminal analysis to assure the safety of their product. The objectives of the three-stage programme are summarized in Table 1; also shown are the agencies responsible for completing each stage and the estimated duration of each stage. Training commenced in Thailand in August 1984 and in Indonesia in November. It is proposed to commence the programme in Malaysia and the Philippines during 1985.

1.1 HACCP Control for Food Manufacture

There are two reasons to apply the HACCP concept to the food manufacturing industry. The traditional control methods achieved through staff education and training, inspection of facilities and operations and microbiological testing or examination have not sufficiently reduced the incidence of food-borne disease (WHO/ICMSF, 1980), and traditional methods are not cost effective. The WHO/ICMSF working party in its 1980 report on the application of the HACCP concept in developed and developing countries defined hazards as including "contamination of food with unacceptable levels of food-borne disease causing micro-organisms and/or contamination with spoilage organisms to the extent that hazards occur within the expected shelf life or use of the product". A critical control point (CCP) was defined as "a location or a process which, if not correctly controlled, could lead to contamination with food-borne pathogens or spoilage micro-organisms or their survival or unacceptable growth". The latter definition is consistent with the assertion of Ito (1974) that "microbiological critical control points must indicate those points where lack of control may cause a potential health hazard due to the presence of an organism of public health significance". In

each of these definitions the emphasis is on protection of public health; however, some manufacturers broaden their interpretation of a critical control point to include factors that if not controlled might effect the marketability of the product. It is believed that by adopting the more stringent (public health) approach to CCPs, the application of the HACCP concept to the manufacture of low-acid canned foods has the prevention of botulism and its primary objective. Thus the distinction between the HACCP and the traditional approach becomes clear; the former relies on in-process tests "to assure that all factors necessary to the application of the established safe processes have been adequately controlled" (WHO/ICMSF, 1980), while the latter relies on terminal analyses to demonstrate the adequacy of the process.

There are three elements involved in the employment of HACCP in process control:

- (i) Assessment of the hazards associated with the manufacture of a food product.
- (ii) Identification of critical control points (CCPs) to control hazards.
- (iii) Procedures to monitor the manufacturing process at critical control points.

1.2 Review of Training Programme in Thailand

Lectures and pilot plant exercises familiarized participants with techniques for identifying and evaluating performance at the critical control points shown in Table 2. Following this, operations in two commercial canneries were reviewed. (Similar reviews for the entire canned fish export sector are proposed in the second stage of the programme.) At the completion of each review manufacturers were provided a confidential report evaluating cannery performance in accordance with the HACCP concept; this enables regulatory authorities and canners to check for compliance with the United States regulations (21 CFR Part 108 and 21 CFR Part 113). Contained in the regulations are the criteria used to assess the adequacy and control of low-acid canned food manufacturing processes; these make clear to prospective suppliers the grounds on which permission to export to the United States will be withheld. Compliance with import regulations must therefore be viewed as mandatory for those intending to secure access to United States markets. As the regulations go beyond the immediate objectives of the training programme it was not necessary to consider them all in detail, however, extracts of relevance to the Thai course are shown in Table 3. While inspecting canneries, course participants had two objectives:

- to check for compliance with United States import regulations,
- to check that at each CCP manufacturers monitor production, provide permanent production records and provide in-line "corrective-action" control mechanisms.

1.3 Preliminary Results for Stage 2 - Thailand

Preliminary results indicate that in the two canneries surveyed there is evidence that manufacturers are not complying with good manufacturing practice, nor have they implemented an adequate in-line process control system at all CCPs. The results summarized in Table 4 give examples of areas where process control was insufficient. While some absence of records may not be considered to affect product safety, it heads the list of the Food and Drug Administration's most frequently reported deviations from good manufacturing practice. No doubt the rationale is that a manufacturer who implements a complete system of records and can demonstrate that production was in control at all CCPs, will have reduced to a commercially acceptable level the chance of a fatal manufacturing error occurring without detection. Although stage 2 of the programme in Thailand is incomplete, some tentative conclusions can be reported:

- (i) Equipment was adequate.
- (ii) There were numerous flaws in process control systems.
- (iii) There was a need for training and certification of retort supervisors.
- (iv) In some instances there was a need for training for production and quality control personnel.

It is considered that these results, coupled with persistent rejections in the United States of Thai canned seafoods (valued at US\$ 14 million for 1981-83, inclusive), may help provide the incentive for implementation of the HACCP concept for canned fish manufacturers.

2. REFERENCES

Ito, K.A., Microbiological critical control points in canned foods. *Food Technol.*, 28(9):46-8 1974

WHO/ICMSF (World Health Organisation/International Commission on Microbiological Specifications for 1980 Foods), Report of Committee on Hazard analysis critical control point. Geneva, WHO

Table 1

FAO programme for IPFC fish canneries

Stage	Objective	Agency responsible	Duration
1	To train national institutas in principles of HACCP control	FAO	2 weeks
2	To evaluata industry performance using the HACCP approach to thermal process control	National instituta	6-9 months
3	To review industry data and conduct ratort supervisor training and certification course	FAO	2 weeks

Table 2

Checklist of critical control points (CCPs) for canned fish manufacture

Raw and packaging material quality

Product temperature/time of praprerstion

Can washing

Filling temperature

Filling waight (liquid to solid ratio if applicable)

Container size and seam adequacy

Retorting (vent, process time, temperature and F_0 value; cooling technique)

Plant sanitation and cooling water chlorination

Line damage

Transport and storage conditions

Table 3

Extracts from Food and Drug Administration regulations
for thermally processed low-acid canned foods

Register establishment, equipment and products

File scheduled process (listing all critical control points)

Confirm that process complies with scheduled process

Report process deviations, spoilage and contamination when safety of distributed goods implicated

Prepare product recall plan

Provide approved supervisor training for retort operation and can double-seam analysis

Maintain records for three years to show conclusively that product could not be hazardous

Table 4

Preliminary results of cannery reviews (Thailand - stage 2)

Criteria evaluated	Acceptability	Examples of errors
Production monitoring	Unacceptable (2/2)	Poor control of product temperature during preparation. No cooling water chlorination. Inadequate cooling. No sanitation
Specifications at CCP's	Unacceptable (2/2)	Retort operating procedure. Cooling time and maximum temperature of cooled can. Cooling water chlorination level. No sanitation programme.
Adequacy of target F_0 values	For safety - acceptable (2/2) For prevention of spoilage - unknown (2/2)	- Raw material quality not monitored nor related to thermal process adequacy
Retort supervisor training and certification	Unacceptable (2/2)	Inadequate cooling. Manual handling wet processed containers
Adequacy for processing records	Unacceptable (2/2)	No thermographs No retort log sheets

Figures in parentheses indicate proportion of canneries reviewed belonging in each category

HANDLING REQUIREMENTS FOR THE EXPORT MARKETING
OF LIVE NEW ZEALAND GREEN MUSSELS

by

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ABSTRACT

Ferma canaliculus, the New Zealand green mussel, is a native shellfish of New Zealand which is cultivated on surface longlines. If the correct handling procedures are adhered to, New Zealand green mussels can be kept alive and maintain their high eating quality for up to 10 days after harvesting. The importance of correctly icing mussels immediately after harvest and maintaining this chilled state is the most important factor in ensuring the supply of high quality live mussels to the consumer. Nothing can replace correctly chilling live mussels under a ceiling of melting ice - other procedures play a supporting role only. Once mussels have entered the "cold chain" begun by the harvester, it is the responsibility of each person who is subsequently involved with the product to ensure that the correct handling and storage procedures are observed to prevent temperature fluctuations. This includes processors, exporters, brokers, distributors, restaurants and retail outlets. The "cold chain" must be maintained from harvest to the point of sale. Utilizing the correct handling requirements, live New Zealand green mussels are now being successfully exported from New Zealand to restaurants throughout the United States, Hong Kong and French Polynesia.

1. INTRODUCTION

Ferma canaliculus, the New Zealand green mussel, is a native shellfish of New Zealand. Farming of the green mussel began in the early 1970s and has now developed into an expanding industry in several locations around New Zealand. During cultivation, mussel spat is seeded onto vertical ropes attached to surface longlines. The longlines are carefully and regularly maintained and the mussels culled until they reach a harvestable size. Production, which is now 10 000 t (22 million pounds) of shell weight product annually, is increasing and is expected to double in the near future.

In recent years the exporting of seafood, fresh chilled by air freight, has become an important part of the New Zealand fishing industry. It is in this segment of the industry that realization of the positive relationship between quality and price is occurring.

Unlike other seafood which is exported fresh chilled by air freight from New Zealand, the green mussel has to be exported alive. This introduced more complex handling and storage procedures to assure that the green mussel remained alive and of high eating quality.

This paper examines the handling requirements which are necessary to successfully export chilled, live, New Zealand green mussels. The paper deals with the United States market which is the major market for New Zealand green mussels.

2. SPOILAGE PATTERNS

The spoilage characteristics of the New Zealand green mussel have been studied by Boyd and Wilson (1978) and found to follow a definite pattern. During storage there is a loss of fresh odour, development of off flavours, and a change in texture. All these changes occur while the shellfish is alive.

Quality grades have been supplied in order to give some quantitative measure to this subjective method of quality assessment. A score sheet outlined in Table 1 is used to assess the freshness of green mussels by scoring samples for odour, texture and flavour on a scale from 5 for very fresh mussels to 0 when almost inedible.

3. POST-HARVEST HANDLING

The rate of loss in the quality of live mussels depends mostly on the temperature at which the mussels are stored (Boyd and Wilson, 1978).

The loss of flavour of mussel shellstock after removal from the water is shown in Figure 1. Mussels that had been packed in ice died very quickly and showed the most rapid loss of flavour. If the quality level acceptable for processing is established at grade 3, the point at which definite off-flavours begin to appear, then iced mussels have a shelf life of only two days.

For mussels held at ambient temperatures (15°-18°C), the first mortality occurred after four days. Noticeable loss in flavour occurred after only two days and the minimum quality grade of 3 was reached after three days. Chilling the mussels between 5° and 7°C extended the time before flavour losses were detectable to three and a half days; they became unacceptable after five days.

Mussels held under a ceiling of melting ice showed the largest increase in shelf life. The mussels are packed into well draining fish cases, a layer of clean secking or stockinette is placed over the mussels and this is covered with a good layer of ice. The ice does not come into direct contact with the mussels and cools them to between 2° and 4°C. It is important that the ice does not come in direct contact with the mussels and that the temperature does not fall below 0°C. At 0°C the mussels die and the shelf life is reduced to one day. When chilling by this method was delayed for 24 h after harvest, the quality remained above grade 3 for eight and a half days and the first mortality was observed after nine days. When chilled four hours after harvest, the mussels remained alive and above grade 3 for 14 days (Boyd and Wilson, 1978).

4. QUALITY STANDARDS

Live New Zealand green mussels for the United States market should ideally arrive at the market place with a grade of no less than 4 for cooked flavour. Any further loss in flavour would mean that due to the high cost of airfreighting, the price received would be insufficient to provide a worthwhile return. According to Figure 1, this would indicate a maximum period of 8-14 days between harvesting and consumption. This would seem to be adequate, taking into account one day for harvesting and one day for the mussels to be transported from New Zealand to the United States. Additional time involves transportation of the live mussels from the harvester to the fish-packing house, packing and transportation to Auckland or Christchurch airport.

5. HANDLING REQUIREMENTS

To verify that the research performed by Boyd and Wilson could be used to send commercial shipments of live mussels to the United States, a shipment of live green mussels was monitored from harvesting and packing in New Zealand to the buyer in the United States.

Live mussels were harvested, transported to the fish-packing house under a ceiling of melting ice and packed (Figure 2) as detailed in the Code of Practice for Mussel Processing (Merwick, 1984).

Information was collected both in New Zealand and the United States by Fishing Industry Board Food Technologists inspecting polystyrene boxes of live green mussels, and recording the data on checklists. The checklists recorded labelling details, temperature, integrity of packaging materials, condition of mussels and an organoleptic evaluation of five samples using Boyd and Wilson score sheet (Merwick, 1983).

On arrival at Los Angeles International Airport, the polystyrene boxes were inspected and the temperature of the live mussels was found to be within the recommended range of between 2° and 4°C (Figure 3).

Ten polystyrene boxes were assessed on arrival, then taken to a wholesaler where they were re-iced and stored in the chiller (Merwick, 1984). The boxes were monitored to ensure that ice was replenished when necessary. Figure 4 shows that after eight days, live mussels had a flavour score of 3.5.

Further samples of polystyrene boxes were assessed at Los Angeles International Airport, re-sealed and monitored through the distribution chain. These samples were not re-iced at any stage. Figure 5 shows that if polystyrene boxes of live mussels are not maintained under chilled conditions (2°-4°C) after arrival in the United States, then cooked flavour is below grade 3 after only four days.

This shows the importance of chilling and maintaining this chilled state throughout the distribution system. Only live green mussels which have been chilled to 2°-4°C under a ceiling of melting ice and maintained in this temperature range will have a flavour score above grade 3, 8 days after harvesting. Any break in the "cold chain" will dramatically reduce the shelf life of the live mussels.

It is not an easy task to maintain the live mussels at/or between 2° and 4°C from harvester to airport, but it is possible and it is controllable. The more difficult stage is from airport to market-place where the control is in the hands of people who have no interest in the product.

Polystyrene boxes may be left outside without refrigeration for many hours during transit in places where temperatures are much higher than in New Zealand. If live mussels are not properly chilled and adequately packed with ice for the journey, they will arrive at the market-place in a poor condition.

The "cold chain" must be maintained once the live mussels have reached the United States. If the mussels are not placed under a ceiling of melting ice after arrival, the high quality shelf life will be halved.

Utilizing the correct handling requirements as detailed in this paper, live New Zealand green mussels are now being successfully exported from New Zealand to restaurants throughout the United States, Hong Kong and French Polynesia.

6. CONCLUSION

The procedures required to produce high-quality, live, New Zealand green mussels are now well known following research by Boyd and Wilson. These procedures primarily involve the achievement and maintenance of an effective "cold chain", coupled with careful handling.

The basic requirements are that live mussels must be chilled to between 2° and 4°C under a ceiling of melting ice as soon as possible after harvest. Once chilled, live mussels must be held at/or between 2° and 4°C during all subsequent handling and transportation stages until the mussels reach the buyer in the United States.

The importance of these procedures lies in the fact that live mussels begin to spoil as soon as they leave the water, but with the appropriate chilling techniques the shelf life can be extended to at least eight days without spoilage characteristics becoming apparent in the cooked flavour.

Extension of the first few days of shelf life is most important for the United States market where only good quality live mussels are acceptable. Here, proper attention to handling and chilling can mean the difference between high quality or low quality product for live mussels of the same age. The extra shelf life gained can also be used to allow processors, exporters, brokers and distributors greater flexibility with respect to planning the despatch of export shipments.

7. REFERENCES

- Boyd, N.S. and N.D.C. Wilson, Handling and processing of raft-farmed mussels. Proc. IPFC, 16(3): 1978 354-8
- Mervick, J.C., Report on technical visit to the United States to study and advise on handling, 1983 transport, distribution and storage of live green mussels. Wellington, New Zealand Fishing Industry Board
- _____, A code of practice for mussel processing. Wellington, New Zealand Fishing Industry Board 1984

Table 1

Mussel score sheet

Cooked odour

Score

5	Fresh mussel, salt water, little odour
4	Mild, buttery, milky, thick smell
3	Slightly sweaty, old socks
2	Rubbery, strong sweaty smells
1	Bland, rubbery pumpkin
0	Faecal, vomit, nauseating

Cooked texture

Score

5	Soft and juicy
3	Drier, juice runs out on chewing
0	Tough and rubbery

Cooked Flavour

Score

5	Characteristic mussel flavour, sweet
4	Slight fishy, briney sweet
3	Strong briney, seawater aftertaste, scallop
2	Strong mussels, slightly sweaty
1	Strong mussels, sour aftertaste, cabbage
0	Difficult to taste, nauseating

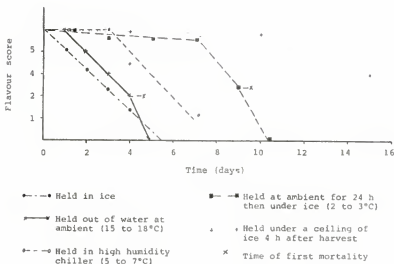


Figure 1 Flavour changes in mussels held under storage conditions

Source: Boyd and Wilson, 1978

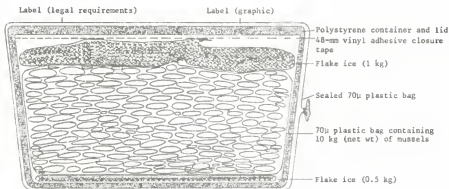


Figure 2 A polystyrene box of live chilled mussels

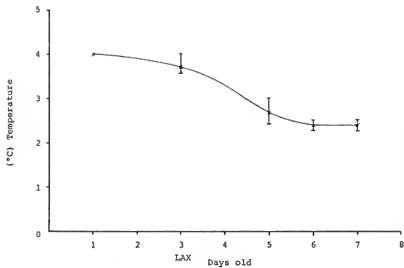


Figure 3 Temperature of live mussels in New Zealand and United States

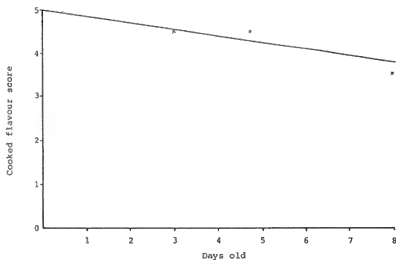


Figure 4 Graph of cooked flavour vs time for properly handled live mussels

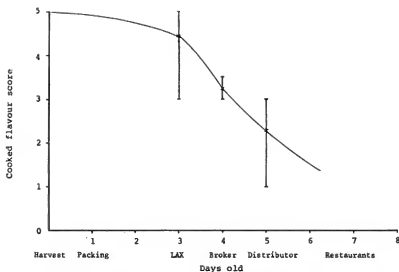


Figure 5 Graph of cooked flavour vs time for polystyrene boxes of live mussels which were not re-iced in the United States

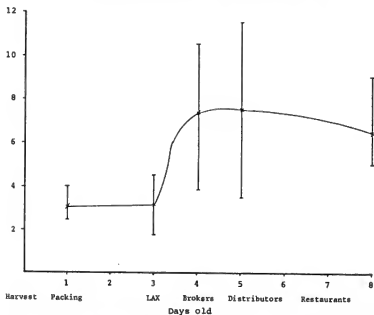


Figure 6 Graph of temperature of time for polystyrene boxes of live mussels which were not re-iced in the United States

THE USE OF ENZYMES IN PROCESSING OF MARINE FOOD PRODUCTS

by

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ABSTRACT

Examples are given of the use of enzymes as aids in processing. Enzymatic deskinning of herring has been accomplished with an acid protease isolated from cod stomachs. Squid is being deskinmed commercially with an enzyme from papaya latex. A process for removing connective tissue from roa sacs with an enzyme from fish intestines is being commercialized while a processing line has been developed to separate oil from muscle cells of minces.

Fish silage and sauce production are two current examples of processing fish by the action of enzymes, in these cases the enzymes being already present in the fish itself (Raa and Gildberg, 1982; Raa, Gildberg and Ström, 1983). Pepsin and other commercially available enzymes are also in use to speed up the solubilization of fish waste for the production of milk replacers for lambs (Soliman and Ørskov, 1976). Thasa ara, however, very crude processes where the final product is a soluble mixture of amino acids, peptides, proteins, fatty acids, etc. The deliberate use of enzymes, indigenous or added, as a more specific tool in fish processing is not so common. However, during the last couple of years we have had considerable success with dissecting and separating fish tissues by controlling the pattern of autolysis and using enzymes to facilitate the degradation of certain tissues.

Autolytic solubilization of fish tissues *post mortem* is an extremely complex biochemical process which results from the combined action of a variety of different enzymes on many equally different substrates (Gildberg, 1978). The process is superimposed by enzyme inhibitors present in certain tissues, and the food status and overall physiological condition of the fish at the moment of catch (Gildberg and Raa, 1980; Hjelmeland and Raa, 1980; Hjelmeland and Raa, 1982; Hjelmeland, 1983). It may therefore appear to be a hopeless task to control and direct these processes so that tissues can be biochemically dissected and separated. Nevertheless, mechanistic studies of the autolytic processes have suggested ways to achieve this.

The skin and muscle of fish differ fundamentally in chemical structure and consequently in solubility as a function of pH and temperature and in susceptibility to enzymatic degradation (Gildberg and Raa, 1979). Due to these differences it is possible to deskin a fish biochemically by careful control of pH and temperature and by simultaneously adding enzymes which attack the skin selectively under chosen conditions.

Enzymatic deskinning of herring is one example of biotechnological processing (Joakimsson, 1984). Whole herring is given a short wash in diluted acid which denatures the surface collagen and strips off bacteria. Subsequently, the herring is subjected to the action of an acid protease isolated from the stomach of cod. After a "acidified incubation period and temperature the tough skin and shells can be washed away with running water, leaving an intact silvery lining on the fillets. The deskinmed herring can now be filleted and immediately put in a salt bath containing enzymes which render the fillets "mature" after one week.

Deskinning of squid is another example of enzymatic removal of tissues which otherwise can be removed only by mechanical and manual means. For human consumption of squid it is essential to remove the rubbery skins from both inside and outside the squid body and from the tentacles. This can be done manually but it is indeed a terrible job and constitutes the major cost of processing squid for the high price markets in Europe. The key to solving the deskinning problem was found when it was discovered that an enzyme present in the squid itself could be activated and thus render the rubbery skins completely free of any tensile strength. It was also found that a minor enzyme component present in papaya latex can do the same, even at temperatures around zero (Raa and Nilsen, 1982).

A completely new processing line has been set up on the basis of this discovery, and the Norwegian fish processing industry has already had notable success on the European delicatessen market with the "skinless white squid". Up to now, several hundred tons have been produced and sold by

Norwegian factories which have adopted the new process, which has a high production capacity at a very low cost, and new producers are currently being taught how to use the process.

Removal of roe from ovary tissues is a third example of enzymatic processing. It is a problem in practice to get out undamaged roe particles from the roa sac when producing fresh roe for the delicatessen market. The mechanical methods used to squeeze out the roe from the roa sac of salmon, trout, herring, etc., are unsatisfactory due to low yields and a high level of damage and denaturation of individual roe particles. An enzyme has been isolated from fish intestines which very quickly releases the roe particles from the connective tissues in the roa sac, even in icy water (Raa, Melvik and Joakimsson, 1983). The remaining connective tissues can be collected with a fork and the roa particles washed in a rotasieve and drained. After a successful pilot production with positive market response, this process is now being introduced in the Norwegian fish processing industry.

Fish mince production from oily fish is a fourth example of biochemical processing (Eide, Børresen and Strøm, 1982). In principle it resembles the deskinning of herring, but involves the complete separation of oil from the muscle calls and subsequent aggregation to a lean (deoil) muscle mince. The theory and practice for this process has been described elsewhere (Strøm and Raa, 1982). A complete processing line has been developed in Tromsø by the Research Institute of Fishery Technology (PTFI) and this process (Norwegian patent No. 144.727) is also being implemented by private industry.

Enzymes in fish and marine invertebrates differ significantly from their counterparts in warm blooded animals and offer distinct advantages for certain applications. Although this, strictly speaking, is outside the scope of this paper, it may be appropriate to draw attention to such facts as:

- Fish pepsins resemble the warm blooded cathepsin D and have remarkable stability (Gildberg and Raa, 1983). Fish pepsins are present in large quantities in fish guts (1 g dry/kg wet) and they are easy to purify and substitute chymosin favourably.
- Trypsin and chymotrypsin from fish have quite different stability levels according to pH and temperature than those from their counterparts from warm blooded animals (Hjelmeland and Raa, 1982) and their solubility characteristics open up certain noteworthy applications outside the fishing industry.

REFERENCES

- Eide, O., T. Børresen, and T. Strøm., Minced fish production from capelin. J. Food Sci., 47:347 1982
- Gildberg, A., Proteolytic activity and the frequency of burst bellies in capelin. J. Food Technol., 1978 13:409
- _____, Autolysis of fish tissue - general aspects. Thesis, Institute of Fisheries, University of Tromsø, Norway, 112 p. 1982
- Gildberg, A. and J. Raa., Solubility and enzymatic solubilisation of muscle and skin of capelin (Mallotus villosus) at different pH and temperature. Comp. Biochem. Physiol. (B. Comp. Biochem.), 63:309-14. 1979
- _____, Tissue degradation and belly bursting in capelin. In Advances in fish science and technology, edited by J.J. Connell. Farnham, Surrey, England, Fishing News (Books) Ltd., pp. 255-8 1980
- _____, Purification and characterisation of pepsins from the arctic fish capelin (Mallotus villosus). Comp. Biochem. Physiol., (A. Comp. Physiol.), 75(3):337-342. 1983
- Hjelmeland, K., Protease inhibitors in the muscle and serum of cod (Gadus morhua); isolation and characterisation. Comp. Biochem. Physiol., (B. Comp. Biochem.), 76:365-72. 1983
- Hjelmeland, K. and J. Raa., Fish tissue degradation by trypsin type enzymes. In Advances in fish science and technology, edited by J.J. Connell. Farnham, Surrey, England, Fishing News (Books) Ltd., pp. 456-9. 1980
- _____, Characteristics of two trypsin type isozymes isolated from the arctic fish capelin (Mallotus villosus). Comp. Biochem. Physiol., (B. Comp. Biochem.), 71:557-62. 1982
- Joakimsson, K., Enzymatisk avskinning av sild (Clupea harengus) Thesis, Fishery chemistry, 1984 University of Tromsø, Norway, 54 p.

- Raa, J. and A. Gildberg., Fish silage: A review. CRC Crit.Rev.Food Sci.Nutr., 16:383-420.
1982
- Raa, J. and K. Nilsen, Norwegian patent nr. 150304-A23L 1/325
1982
- Raa, J., Gildberg, A. and T. Ström., Silage production - theory and practice. In Upgrading waste
1983 for feeds and food, edited by D.A. Ledward, A.J. Taylor and R.A. Lawrie. London,
Butterworth, pp. 117-32.
- Raa, J., G. Molvik, and K. Jonkimsson., Norwegian patent appl. 834005
1983
- Soliman, H.S. and E.R. Ørskov, Utilisation of fish-protein hydrolysate for artificial rearing of
1976 lambs. Proc. Nutr. Soc., 35:91A
- Ström, T., and J. Raa., A new processing method for small pelagic fish. Infofish Mark.Dig., 3:24-7
1982

VACUUM POUCH PRODUCT DEVELOPMENT IN SRI LANKA

PART I: PROCESSING

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ABSTRACT

Studies related to the processing technology and storage characteristics of haat sterilized, vacuum packed fish were carried out with the view of introducing the product as a substitute for imported canned fish. The present study deals with the processing aspects related to herring (*Amblygaster sirm*), sardines (*Sardinella longiceps*), tilapia species, milkfish (*Chanos chanos*), tuna and flying fish (*Exocoetus* sp.) in chilli/tomato sauce and brine. Laminated-O-Nylon, C-polypropylene was used as the packing material. The shelf life and some organoleptic aspects of the product during storage of vacuum packed herring and sardines is discussed.

1. INTRODUCTION

The coastal, offshore and deep sea fishing contribute to over 80% of the fish catch in Sri Lanka (Etoh, 1983) of which 67,982 tons (1980) and 63,436 tons (1981) consisted of sardines and sardine-type fish varieties (Ministry of Fisheries Sri Lanka 1980-81). The demand for fresh marine fish will increase in the years to come while the availability of fresh marine fish remains limited. Vacuum sealed retort pouched fish packs, using sardine-like small fish was developed as a means of utilizing seasonal gluts, as well as providing a substitute for fresh fish when necessary. Adams and Ottwell (1982) state that the retort pouch can provide a processed product superior to current canned items.

Vacuum packed retort pouched trench sardines were designed as a "ready to eat", "boil in the bag" fish product.

2. RETORT POUCHING MATERIAL

Retort pouches are currently made from various types of material. The packaging material stated below was used in the present study.

Type 1

Laminated with-O-nylon-C-
polypropylene
(transparent)

Type 2

Laminated pouch casted
polypropylene (70 u), polyester
(12 u) and aluminum (12 u)

3. VACUUM POUCHED PREPARATIONS

Raw material for retort pouching ideally consists of species belonging to *Clupeidae* family such as *Sardinella* sp., trench sardines (*Amblygaster sirm*), flying fish and milkfish (*Chanos chanos*). Any type of small fish with the absence of sharp bones are suitable for processing. Tuna and skipjack were also used in the processing of chunks and flasks.

Listed below are some vacuum pouched preparations processed and stored at room temperature - 28°C.

Brine	Chilli	Tomato	Oil
Sardines	T. sardines	T. sardines	<i>Sardinella</i>
T. sardines	Flying fish	Milkfish	<i>longiceps</i>
Skipjack			
Tuna			

Proximate composition of some fish products are given in Table 1. All the above mentioned were processed Type 1 pouches, which are transparent in natura and cheaper than type 2.

Table 1

Proximate composition of some vacuum packs

	(%) Protein	(%) Moisture	(%) Oil	(%) FFA
Tranchad sardina/Brine				
<i>A. sirm</i>	21.8	72.0	2.7	12.3
Sardines/Brine				
<i>Sardinella</i> sps.	18.7	74.5	3.6	19.5
Skipjack/Brine				
<i>Katsuwonus pelamis</i>	30.7	62.9	1.1	19.4

3. PROCESSING SMALL VARIETIES OF FISH

The sequence of processing is given in Appendix 1, where the dressed fish is brined for 7-15 min. in a supersaturated brine solution, washed lightly, filled into vacuum pouches and sealed. Chilli or tomato paste (Appendix 3) was added after the filling of fish, if a spiced preparation is required. All packs were vacuum sealed in a BIBUN (BIBUN Company Japan) vacuum sealer.

The sealed pouches were arranged in layers in a retort, and sterilized at 121°C for 45 min, after which cooling was carried out under pressure. Fo velums for vacuum packed retorted fish ranged from 23 min (brine) to 26 min (sauce), but heat treatment of 45 min gave products of better organoleptic acceptability (similar to canned fish). No pre-cooking such as steaming, drying or pre-boiling prior to retorting was necessary in the processing of these products. Uniform thickness of packs was maintained while sealing in order to obtain uniform heat distribution to all packs during sterilization.

4. PROCESSING OF SKIPJACK AND TUNA

The sequence of processing is given in Appendix 2. The fish are dressed as stated earlier and a horizontal cut was made along the red meat after which the fish were steamed at 100°C for 1-1 1/2 h. Once steamed they were dried at room temperature for 18-20 h and then cut into steaks and packed into pouches with 0.1% salt. Sealing and retorting was carried out as mentioned earlier.

5. ACCEPTABILITY AND STORAGE OF THE PRODUCT

Vacuum packed/sterilized fish products being a new type of product in Sri Lanka, resulted in studies carried out in order to observe the reaction among consumers/taste panelists to the organoleptic and visual acceptance of these products. It was observed that the use of low quality fish as in the case of *Trachurus* resulted in a product with excessive liquid and consequent disintegration of pieces. In *Sardinella* sp. disintegration as well as yellowing of oil and the development of rancidity was observed.

A study was carried out to determine the storage and acceptability of vacuum packed retorted tranch sardines packed in chilli. Two batches of fish of slightly varying chilli paste formulations (Appendix 3) were tested regularly (50-day intervals) using a 5 point scale by 10 panelists. Quality of fish was defined as "good" and "bad" for convenience of description.

Formulations used - chilli paste only

Chilli paste	Chilli paste	Chilli paste	Chilli paste
+ 0.5%	+ 1.0%	+ 2.5%	+ 7.5%
citric acid	malic acid	dilute tomato rind	commercial vinegar

On observing Figure 1, the fish packs processed from "good" quality fish were found to be acceptable with overall scores ranging from 3.5 - 4.0 initially to 3.2 - 3.6 finally (at 150 days), where not as much variations in scores was observed in "bad" quality fish. A shelf life of 150 days could be recommended for vacuum packed retorted fish (type 1 pouches) where as a longer shelf life could be achieved using type 2 pouches. Care is necessary in handling pouches at all stages of processing due to the possibility of pinhole damages, which causes immediate bulging and spoilage.

A marketing trial carried out in order to obtain a broader view of most Sri Lankan consumers indicated the acceptability of the product, mainly since it was formulated to suit the Sri Lankan palate, the visibility of the product in the pouch and its instant nature.

Retort pouching could provide a suitable substitute for fresh or canned fish, provided suitable and safe conditions are employed in processing the product. A shelf life in excess of 3 months is safely recommended, which could be further extended if type 2 pouches are used.

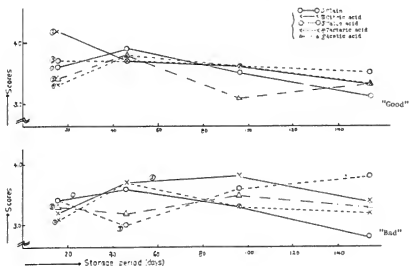


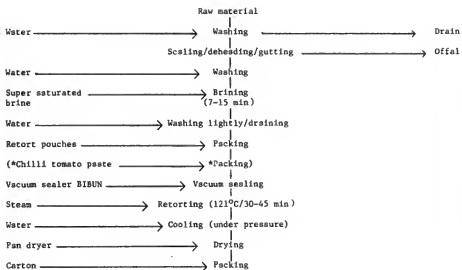
Figure 1 Changes of overall sensory evaluation scores during storage

6. REFERENCES

- Adams, J.P. and W.A. Otwell, Suitability of seafoods to retort pouch processing. In Proceedings of the Seventh Annual Tropical and Subtropical Fisheries Technological Conference of the Americas, Jan. 11-4, 1982
- Etoh, S., Manufacture of vacuum pouch from small varieties of fish like cardines. Colombo, 1983 Sri Lanka, Institute of Fish Technology, Project Report

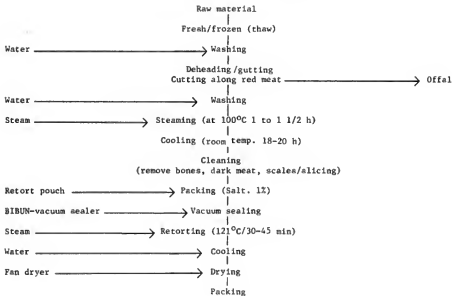
APPENDIX 1

Method of processing/vacuum
packed retorted - small fish varieties



APPENDIX 2

Method of processing/vacuum
packed retorted - Tuna/skipjack



APPENDIX 3

Some formulations used
in preparations

Chilli Paste (per 100 g fish)

Chilli powder	1.4
Salt powder	0.7
Sugar	2.4
Ginger (fresh)	0.3
Garlic (fresh)	0.6
Onions (fresh)	1.6
Water	2.7
Citric acid	0.04

Tomato Paste (per 100 g fish)

Tomato ^{1/}	37.0
Salt	1.2
Sugar	4.0
Onion (fresh)	2.6

Blend to a smooth paste

Blend to a smooth paste

All packs contain a net weight of 250 g (225 g) fish: around 25 g paste

^{1/} Obtained by blending/straining and drying fresh tomato to obtain a concentrate

HEAT RESISTANCE OF SPOILAGE BACTERIA
IN RELATION TO THERMAL PROCESSING OF CANNED FISH SAUSAGES

by

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ABSTRACT

A case of spoilage was observed in a batch of canned fish sausages in natural casing, which were subjected to a thermal process equivalent to $F_{250} = 5.2$ min. Tests showed that the causative organism was a highly heat-resistant, spore-forming *Bacillus*, of the 'flat-sour' type. Experiments revealed that the spores had thermal death times (TDT) of 90 min, 24 min and 8 min at 100°, 115.6° and 121.1°C, with a D-value of 4 min at 115.6°C and Z value of 10.4°C. Analysis of raw materials showed that the starch used for the sausages was highly contaminated with flat sour spores. Since fish sausages are liable to high temperatures, it is not feasible to adopt canning processes much more severe than $F_{250} = 5$ or 6 min and, therefore, it seems imperative that starch and spices used as ingredients should be free from highly heat-resistant 'flat-sour' bacteria.

1. INTRODUCTION

Fish sausage, a popular product of Japan, has not been commercially accepted in India because no local substitutes for the chemical preservative 'AF-2' (furyl furamide) and synthetic casing of 'saram' are available (Yermal, 1970). Studies were carried out to assess the possibility of using locally available raw materials for fish sausage manufacture and to assess the overall effects on quality and shelf life (Krishnaswami, Rudra Setty and Revankar, 1964; Krishnaswami and Rudra Setty, 1966; Krishnaswami and Patel, 1972). Since these studies failed to indicate any satisfactory alternatives for the casing and preservatives, a method of canning fish sausages in natural casing (sheep guts) was developed by Saralaya and Bhandary (1977) with a view to eliminate the need for preservative and synthetic casing. They suggested a thermal process of $F_{250} = 5.2$, based on heat penetration studies and heat resistance of a strain of *B. circulans* reported by Tanikawa (1971) as being the most heat-resistant organism associated with fish sausage spoilage. In a later study, Saralaya *et al.* (1980) discussed the effects of thermal processing on quality characteristics of canned fish sausages and inferred that the above process yielded satisfactory products in small containers.

However, spoilage of fish sausages thermally processed by the above method was encountered. A study on the heat resistance of the causative organism is the subject of this paper.

2. MATERIALS AND METHODS

Twenty cans of spoiled fish sausages were sampled for analyses, eight for general examination of cans and 12 for microbiological tests. All the cans were internally coated with sulphur-resistant lacquer, having dimension 301 x 206 mm and net contents of around 165 g of dry packed fish sausages in natural casing.

2.1 General Preliminary Examination of Product

The preliminary examination was conducted to determine the general type of spoilage and its cause according to the method suggested by Hersom and Molland (1968). External and internal condition of cans, degree of vacuum, condition of contents and quality of double seaming were examined in detail and recorded. Microscopic examination of direct smears from aseptically opened cans was carried out. Cultures were made on agar plates, glucose tryptone broth, thioglycollate broth and pea infusion broth. Tests were conducted to determine aerobic and anaerobic growth on pasteurized as well as unpasteurized tubes and incubated at both 37° and 55°C. The microbiological media used were prepared according to APHA (1966).

2.2 Isolation, Identification and Preparation of Spores

Selected colonies from agar plates were subcultured, and identified to genus level using the method of Somers (1968), with particular attention to species of *Bacillus*. Spores were obtained

on nutrient agar slants to which a few whole green peas were added to each tube in order to obtain good growth after incubation at 55°C for four days. Spore suspensions were prepared by breaking clumps, filtering and pasteurizing to eliminate vegetative cells. The suspensions were diluted to a final concentration of 400 to 500 million spores per millilitre which constituted the stock spore suspensions.

2.3 Determination of Heat Resistance

The stock suspensions held under refrigeration (+20°C) were further diluted to give one million spores per millilitre using sterile water. The concentration was verified by plating on agar. Thermal death time (TDT) determinations were made at 110°C, 115.6°C and 121.1°C, following the TDT tube technique described by Somers (1968). The heating time intervals were 5 min at 110°C, 2 min at 115.6°C and 1 min at 121.1°C, as determined through preliminary trials. The survival and death times were recorded and plotted for obtaining the straight line semi-log curve, from which Z value was calculated. For confirming the TDT value, decimal reduction time of 'D-value' was estimated according to the method described by Stumbo (1973), based on the death rate (or survivor) curve of the organism at 115.6°C.

2.4 Examination of Raw Materials and Ingredients

Samples of fish, natural casings, starch and spice extracts were tested for total plate counts, spore counts and flat sour spores, following standard procedures. The fish and natural casings were obtained from local market. 'Old' samples of starch were those in stock for about one year, while the 'new' samples were those which had been held for less than a month. All the samples were similar to those used in the preparation of fish sausages. The spice extracts were oleoresin extracts of high bacteriological quality.

3. RESULTS AND DISCUSSION

The results of preliminary examination of canned fish sausages are summarized in Table 1. Table 2 presents the main findings of bacteriological tests carried out. The thermal death time (TDT) values of the spores are given in Table 3. Z-value of spores can be seen from the semi-log TDT curve of Figure 1. The slope of the death rate curve (Figure 2) shows the D₂₄₀ value for the same spores. Microbiological profile of the raw materials and ingredients of fish sausage are presented in Table 4. Figure 3 indicates the changes in F₂₅₀ values of thermal process given and in the jelly strength of canned fish sausages, in relation to duration of retorting.

It can be seen from the results of preliminary examination of the can and contents that the spoilage case under study is one that is generally classified as 'flat-sour' type. The normal can ends, absence of dents, pin-holing or rusting, practically no gas formation and, above all, definite lowering of pH from the normal 6.3-6.6 to 5.0-5.8, are all evidences which lead to this conclusion (Table 1).

Microbiological examination, showing only one species of spore forming, Gram positive, acid-producing rods, confirm the above finding. The general cause of spoilage in the present case may be attributed to 'underprocessing' or 'process failure', according to the scheme of Herson and Hulland (1968), due to the survival bacteria belonging to the genus *Bacillus*.

The tests show that the spoilage organism under consideration conforms to most of the characteristics of *Bacillus* genus, species of which are classified either as aerobic spore formers or, more often, as facultative anaerobes. Production of spreading colonies on agar plates, showing better growth near the surface in broth culture, preference for media containing starch or carbohydrates (like pea infusion broth) and acid production with resultant lowering of pH in food, all these lead to the above conclusion. The organism isolated prefers thermophilic growth temperatures of 50-55°C, but is capable of growing at lower mesophilic temperatures in the range of 30-37°C. There are many species of *Bacillus* which are generally considered as thermophiles, but can often form strains capable of growing at lower temperatures, as in the case of this spoilage organism (Smith, Gordon and Clark, 1952). Several bacterial species usually considered as 'flat-sour' type of thermophiles can be induced to grow at much lower temperatures. The spoilage bacteria from canned fish sausage may be a strain of such species.

Both thermal death time tests and decimal reduction time (D-value) determinations conducted on the spoilage organism showed that it had a high degree of heat resistance in spore form (Table 3, Figures 1 and 2). The TDT of 8 min at 121.1°C and D₂₄₀ value of 4 min are much higher than those of *C. botulinum* spores and of *B. circulans* (Tanikawa, 1971), but less than those of *B. stearothermophilus*. With such highly heat-resistant spores contaminating the product, it is natural that the thermal process adjusted to F₂₅₀ = 5.2 should fail.

The results of microbiological analysis of fish sausage ingredients in Table 4 showed that the source of contamination was the starch used. Spices can be a good source for flat sour bacteria, but pre-sterilized species and spice extracts are free from these (Table 4). In view of this

finding, the microbiological quality of starch assumes a high degree of significance. Under the circumstances it is relevant to review the thermal process requirements for the manufacture of canned fish sausages.

In the earlier work (Sarataya and Bhandary, 1977) processing based on the heat resistance of *B. circulans* ($F_{250} = 5.2$) was suggested as adequate for preventing spoilage in canned fish sausage. Such processes were also shown to yield acceptable products (Sarataya et al., 1980). It was also reported that the desired qualities of fish sausages, such as colour, texture, resilience and flavour, could not withstand more severe heat treatments. Jelly strength of canned fish sausage decreases with increase in F-value. Jelly strength and other qualities are also known to deteriorate further during storage of the canned product. Considering these facts, it is evident that processes with F_{250} values of over 6-7 min would yield canned products of poor quality. In order to avoid excessive thermal processing starch and other ingredients free from microbial contamination should be used.

In this connection it is interesting to note that the starch samples tested in the present study had high spore counts (Table 4), much higher than the limits prescribed for starch to be used in canned foods (Somers, 1968). It can be readily shown that with a starch having 10 spore/g, a can of fish sausage (165 g with 6% starch) will have about 100 spores/can, and to sterilize a batch of 10 000 such cans, a million spores should be reduced to less than one. This means that processes applied should have F_{250} values of 8 min or more at can centre. If spores of still higher heat resistance (e.g., *B. stearothermophilus*) are present, even such processes would fail. Therefore, it appears reasonable to conclude that starch and spices of very high quality alone can yield canned fish sausages of good quality with low spoilage risk.

4. REFERENCES

- APHA, Recommended methods for the microbiological examination of foods. New York, APHA, pp. 181-8
1966
- Hersom, A.C. and E.D. Hulland, Canned foods; an introduction to their microbiology. London, J. and
1968 A. Churchill Ltd., pp. 306-7
- Krishnaswami, M.A. and J.D. Patel, The effect of certain preservatives on the shelf life of fish
1972 sausages. J.Food Sci.Technol., 9(11):10-2
- Krishnaswami, M.A. and T.M. Rudra Setty, Orange oil as a preservative for fish sausage. Bull.Jap.
1966 Soc.Sci.Fish., 32(11):972-5
- Krishnaswami, M.A., T.M. Rudra Setty and G.D. Revankar, Studies on some species of fish and pre-
1964 servatives in the manufacture of fish sausage. J.Food Sci.Technol., 1(6):64-7
- Sarataya, K.V. and M.H. Bhandary, Studies on the canning of fish sausages. 1. Heat penetration
1977 pattern and thermal process requirements. Mysore J.Agric.Sci., 12(3):479-84
- Sarataya, K.V., et al., Studies on the canning of fish sausages. 2. Manufacture, processing and
1980 quality aspects. Mysore J.Agric.Sci., 14(1):102-8
- Smith, W.R., R.E. Gordon and F.E. Clark, Aerobic Sporeforming bacteria. Agric.Monogr., USDA, (16)
1952
- Somers, I., A laboratory manual for food canners and processors. (U.S. Canners Association).
1968 Westport, Connecticut, AVI Publishing Company, Inc., 336 p.
- Stumbo, C.R., Thermobacteriology in food processing, London, Academic Press, pp. 18-23
1973
- Tanikawa, E., Marine products of Japan. Tokyo, Koseisha Koseikaku Company, 406 p.
1971
- Yermal, J.R., Fish sausage manufacture and future prospects of the industry. Seafood Export J.,
1970 2(5):19-28

Table 1

Summary of results on the examination of spoiled canned fish sausages

Item	Particulars
Name and nature of product	Fish sausage in natural casing, dry pack (without any liquid filling medium)
Container type and size	8 oz, round, cylindrical cans (301 x 206 mm), internally coated with sulphur-resistant (SR) lacquer
Product code	FSD (Fish sausage, dry pack)
Condition of can	
(e) Pin holes or rusting	Absent
(b) Dents	Nil
(c) Buckling or panelling	Absent
Appearance of can (external)	Mostly normal with flat or concave ends, except in one sample which was a suspected flipper. Vacuum positive, low (in flipper can) to cover 22 cm in other samples
Double seam examination	
(a) Visual (external and internal)	Normal at both ends in all cans
(b) Wrinkles	Negligible, considering can diameter
(c) Overlap	Good (> 50% in all cases)
(d) Overall seam quality	Good, no possibility of leakage through seams
Condition of contents	
(a) Colour of solids	Normal. No evidence of fading or discolouration
(b) Appearance	Disintegrated in varying degrees
(c) Texture	Broken down, easily crumbling on touch
(d) Odour	No putrefactive odour
(e) Liquids	2-3 g present in cans, suggesting hydrolysis
(f) pH	Definitely below normal (5.0-5.8)
Can interior	No evidence of lacquer peeling or dissolving. No evidence of sulphide blackening, internal corrosion or detinning.

Table 2

Main findings of bacteriological examination of spoiled canned fish sausages

Test	Findings
Direct smear and microscopy	Rod type bacteria seen - a few to several per field
Gram stain	Positive
Spores	Present, oval, central to subterminal
Cultural and other characteristics	Colonies whitish, circular and mostly surrounded by yellow zone on BCP agar, suggesting acid production; liable to result in spreaders when plates are not overpoured with plain agar layer. Better growth observed in Pe-2 broth than in glucose tryptone or thioglycollate broth. Growth occurs near surface of broth in the tubes. Cultures seem to prefer thermophilic temperatures (50°-55°C), even though growth seen at lower mesophilic (35°-37°C) temperatures. Sugar fermentation and other reactions conforming to those of <i>Bacillus</i> genus. All selected colonies seem to exhibit similar characteristics, suggesting organisms belong to the same species.
Sporulation	Good spore crops could be obtained on nutrient agar slants, with a few green peas added, in four days at 55°C.

Table 3

Heat resistance of spores of spoilage bacteria from spoiled canned fish sausages

Heating temperature (°C)	Time of heating in minutes	
	+ (survival)	- (death)
110	80	90
115.6	22	24
121.1	7	8

Note: Z value from straightline TDT curve based on above values (Figure 1) = 10.4°C

Method: TDT tube (9 x 150 mm tubes with wall thickness 1 mm)

Spore concentration - one million spores per millilitre

Come up time of tubes - 1.5 min (after correction)

Heating medium - sterile water (pH 7.0)

Subculturing medium - Pe-2 broth (with a few peas per tube)

Incubation temperature - 37° and 55°C

Table 4

Microbiological profile of ingredients used in preparing canned fish sausages

Ingredient	Average count per gramme ^{a/}		
	TPC	TSC	FSC
1. Pink perch fish (<i>M. japonicus</i>)	3.8×10^6	2.3×10^3	0
2. Tuna (<i>E. affinis</i>)	1.6×10^5	5.6×10^2	0
3. Natural casing (Sheep guts, cleaned)	7.6×10^3	5.0×10	0
4. Starch (a) Old	2.7×10^5	4.1×10^3	53
(b) New	6.2×10^4	8.3×10^2	9
5. Spice extract	-----	NIL	-----

Nota: TPC = Total Plate Count
TSC = Total Spore Count
FSC = Flat Sour Spore Count

^{a/} Average of four samples in each case

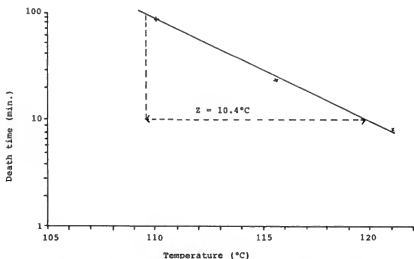


Figure 1 Heat resistance of spoilage bacteria from canned fish sausage

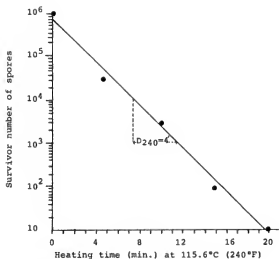
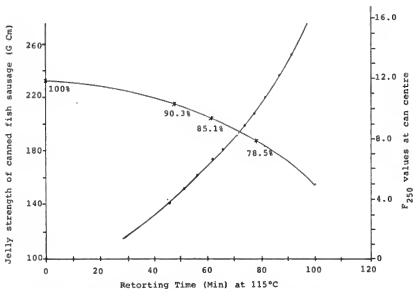


Figure 2 Death rate (survivor) curve of spores of bacteria from spoiled canned fish sausage



(Based on results of earlier work of the authors)

Figure 3 Relationship between retorting time, F_{250} value and jelly strength of canned fish sausage

A PROCESS FOR REDUCTION OF BENZO(A)PYRENE CONTENT
IN SMOKED OIL SARDINE

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ABSTRACT

Samples of oil sardines (*Sardinella longiceps*) were subjected, after salting, to varying conditions of smoking, such as reduction in the combustion temperature, use of smoke filters, control of air supply during smoking and drying after smoking. The products were analysed for 3,4 benzo(a)pyrene content using a Spectrofluorometer - 203. The results showed that by reducing the combustion temperature of sawdust to 300°-400°C, using sawdust with 30% moisture level and smoking at $45 \pm 5^\circ\text{C}$ for $1\frac{1}{2}$ h, followed by smoke processing at $70 \pm 5^\circ\text{C}$ for $2\frac{1}{2}$ h, using filters of mesh size 0.5 to 1 mm and controlling the air supply, it was possible to reduce 3,4 benzo(a)pyrene content of the products nearer to the safe level fixed by the International Union of Pure and Applied Chemistry (IUPAC, 1967). The process also yielded a product of desired colour, taste and acceptability.

1. INTRODUCTION

Smoke-curing of fish by exposure to smoke, generated by burning the wood, is an age old practice and is believed to have developed as an adjunct to drying methods of preservation (Fiddler, *et al.*, 1966). The beneficial effect of smoke-curing is that it imparts desirable flavour and colour to products, contributes substantially to preservation by acting as an effective anti-oxidant and bacteriostatic and bacteriocidal agent (Hess, 1929; Banks, 1952; Gibbons, Rose and Hopkins, 1954; Daun, 1979) and provides a protective film on the surface of smoked products. Smoke-curing of fish is not fully safe, since contamination with potentially toxic substances may occur during processing. These substances are the polycyclic aromatic hydrocarbons (PAH), which are known to be carcinogenic. Among the PAH present in smoked food, the most common is the 3,4 benzo(a)pyrene (Gilbert and Knowles, 1975). Interest has been shown during the last two decades in detecting this chemical, which is also known to be an arbitrary indicator of the presence of carcinogenic PAH in foods (Lijinsky and Schubik, 1964; Daun, 1979). This, along with many other PAH, is formed from thermally generated methylene radicals (CH_2) during pyrolysis of wood and is easily deposited on, or absorbed by, food surface during smoke-curing process (Tilgner and Daun, 1964 and 1970). The deposition or absorption of actual amounts of such carcinogenic compounds in smoked products could be controlled by varying factors including method of smoke generation, form of smoke application, temperature of smoke-curing and other (Potthast, 1978).

An attempt has been made in the present study to establish conditions which would minimize the benzo(a)pyrene contents in smoke-cured oil sardine.

2. MATERIALS AND METHODS

2.1 Smoke-Processing

Fresh oil sardines (*Sardinella longiceps*) caught from the Arabian Sea off Mangalore were used in this study. They were dressed (scaled, gutted and washed), dry-salted and smoked in the smoke chamber by burning the husk of coconut (*Cocos nucifera*), sawdust of dried mango (*Mangifera indica*) and logs of acacia (*Acacia* sp.) in equal proportions. The smoke chamber used was of vertical type, having facilities for generating smoke, recording temperature and relative humidity, unloading and loading of fish (Figure 1 (a and b)).

For smoking, the dressed and dry-salted fish were hung by the hooks in the smoke chamber and subjected to varied smoking conditions, such as reduction in temperature of pyrolysis, variations in smoking temperature and duration of smoking, supply of air and use of filters (Table 1). The dried wood of acacia sawdust and husk were mixed and grouped into small beds of approximately 3 000-4 000 g in the chamber and the temperature of generated smoke was controlled by burning the required number of beds at a time. In all the variables, the sawdust was made damp by mixing tap water to

the extent of 30% before burning in order to reduce the combustion temperature of wood. The combustion temperature was measured by using a pyrometer. Intake of air from the atmosphere during pyrolysis was restricted by regulating the shutter provided at the bottom of the chamber. To filter the smoke, two-layer filters of mesh size $0.5-1 \text{ mm}^2$ were used (Figure 1b). In the control sample, none of the variables was used.

2.2 Extraction and Analysis of Benzo(a)pyrene

After processing, the smoked oil sardines were analysed for benzo(a)pyrene by adopting the procedure of Rhee and Bratzlar (1968). The benzo(a)pyrene content was estimated with the help of spectrofluorometer - 203 under excitation, 305 nm, emission wave length 405 nm, sensitivity 5 and selection XI.

3. RESULTS AND DISCUSSION

The results of the experiment are shown in Table 1. Levels of $11.73 \mu\text{g}$ of benzo(a)pyrene/kg of smoked product were found in the control sample. By using dampened sawdust with 30% moisture restricting the flow of air for burning of wood and use of filters, it was possible to reduce the benzo(a)pyrene content substantially in all the five experimental samples. The product of sample 5, which had $1.6 \mu\text{g}$ of benzo(a)pyrene/kg of smoked product, was not found to be acceptable on account of light yellow colour and rancid taste. The product of sample 4 was found to possess good taste, but did not possess an acceptable colour. The product of sample 3 was found to possess both taste and colour and its benzo(a)pyrene content was $2.4 \mu\text{g/kg}$.

Polyaromatic hydrocarbons are formed from methylene radicals during the pyrolysis of wood and wood products (Tilgner and Daun, 1964 and 1970). Several workers have attempted to bring down the formation of PAH in the smoke by lowering the temperature of pyrolysis (Miller, 1962; Tilgner and Daun, 1964; Potthast, 1975). Reduction has also been obtained by drawing smoke from external smoke generators, using electrostatic filters and liquid smoke instead of traditional kilns (Butz, Kopalava and Pelikanova, 1971; Stainig, 1976). It is also reported that the curing smoke could be made entirely free from benzo(a)pyrene and anthracene contents and related compounds by not exceeding the temperature zone of thermal destruction above 425°C (IUPAC, 1967). Foster and Simpson (1961) have shown that the burning temperature of wood can be reduced by varying the moisture content from 13% to 45% in the bottom and top layers of wood shavings. They also demonstrated that by fitting a duct to the smoke chamber with an electrical precipitator and diffusing screens, consisting of two-five layers of 70 mesh brass wire cloth, the deposit of PAH could be reduced. In the present experiment, a combination of factors like addition of 30% moisture to sawdust to bring down the temperature of pyrolysis, controlled air supply and use of smoke filters were responsible for the reduction of benzo(a)pyrene levels in the smoked products. In addition, the adjustment of duration of smoking, followed by electrical or sun-drying, have helped reduce benzo(a)pyrene levels. By using a combination of these factors it has been possible to reduce the benzo(a)pyrene level to as low as $1.6 \mu\text{g/kg}$.

In general, golden yellow colour and good taste are the important criteria in the consumer acceptance of smoked products (Daun, 1979). Based on these criteria, sample 5 can be considered acceptable, while sample 4 can be adjudged as acceptable but for the light yellow colour; but from the points of taste and acceptability, the product of sample 3 only fulfils the required level of acceptability.

There are several reports in literature linking the PAH to various kinds of neoplasms. The role of PAH in carcinogenesis is well established in a population study consisting of Baltic fishermen (Kaufman, Miranowa and Szobod, 1959). The incidence of neoplasms among the fishermen who ate heavily smoked fish was three times higher than among those who did not eat the heavily smoked fish. The heavily smoked fish is reported to contain an appreciable amount of benzo(a)pyrene and this was responsible for the high incidence of stomach carcinomas among the fishermen (Bailey and Dungal, 1958). Among the more than 20 PAHs and their derivatives which have been found to be carcinogenic, 3,4 benzo(a)pyrene is the most important and is believed to be an indicator of the presence of carcinogenic material in food stuff (Dikun, Lewrobvki and Szmlowska, 1967). The carcinogen, benzo(a)pyrene, has been identified in various broiled, smoked meat, fish and other similar types of products (Bailey and Dungal, 1958; Grimmer, 1966). Therefore, it is necessary that while processing and preserving the fish through smoke process, the benzo(a)pyrene content of smoked product should be taken into consideration and a product with only good taste and colour should be recommended for human consumption.

A sensitivity of $2 \mu\text{g}$ of benzo(a)pyrene/kg of product has been considered to be the safe tolerance level for smoked products (IUPAC, 1967). In the present experiment, the product of samples 4 and 5 only falls below this prescribed level. The product of sample 5 is not acceptable on account of its rancid taste and light yellow colour. The product of sample 4, the benzo(a)pyrene content of which is below that of the IUPAC prescribed limit could be considered as safe and acceptable, the only drawback being its light yellow colour. On the other hand, the product of sample 3 could be considered as the safest and most acceptable from the point of view of taste and colour, even though its benzo(a)pyrene content is slightly more than the IUPAC standard.

It may, therefore, be concluded that by reducing the combustion temperature to $300^{\circ}\text{--}400^{\circ}\text{C}$ by using damp sawdust and smoking the product fish at $45 \pm 5^{\circ}\text{C}$ for $3\frac{1}{2}$ h and at $70 \pm 5^{\circ}\text{C}$ for $2\frac{1}{2}$ h and with the help of smoke filters of 0.5 mm and 1 mm mesh size controlling the air supply, it is possible to get a product of desired taste, colour and acceptability, with its benzo(a)pyrene content nearer to the standards fixed by the IUPAC.

4. REFERENCES

- Bailey, E.J. and N. Dungal, Polycyclic hydrocarbons in Icelandic smoked food. Brit.J.Cancer, 1958 12:348
- Banks, A., The development of rancidity in cold-stored herring and the influence of some anti-oxidants. J.Sci.Food Agric., 3:250
- Daun, H., Interaction of wood smoke components and foods. Food Technol., 5:67-70
- 1979
- Dikun, P.P., A.A. Lawrobnikij and N. Szmulewska, The formation of 3,4 benzopyrene as a result of wood pyrolysis at $300\text{--}400^{\circ}\text{C}$. Vopr.Onkol., 13:3
- Fiddler, W., et al., Composition of hickory sawdust smoke, furans and phenols. Agric.Food Chem., 1966 14:659-62
- Foster, J. and T.H. Simpson, Studies on the smoking process for food. 1. Importance of vapours. 1961 J.Sci.Food Agric., 12:363-73
- Gibbons, N.E., A.H. Rose and J.W. Hopkins, Bactericidal and drying effects on smoking of bacon. 1954 Food Technol., 8:155
- Gilbert, J. and M.E. Knowles, The chemistry of smoked foods: a review. J.Food Technol., 10:245-61
- 1975
- Grimer, G., Carcinogene Kohlenwasserstoffe in der Umgebung des Menschen. Erdol U. Kohle. Erdgas. 1966 Petrochemie, 19:578
- Hess, E.A., The bactericidal action of smoke contrihs. Can.J.Biol.Fish., (4):29 p.
- 1929
- IUPAC (International Union of Pure and Applied Chemistry), Trace substance committee, Food section, 1967 Washington, D.C.
- Kaufman, B.D., A.U. Miranowa and L.M. Szohod, Vopr.Onkol., 5:31
- 1959
- Lijinsky, W. and P. Schubik, Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal broiled meat. Science, Wash., 145:53
- 1964
- Miler, K., Possibilities of curing smoke generation free from 3,4 benzopyrene and 1,2,5,6 dibenzanthracene. Ph.D. Thesis. Politechnika Gdansk, Gdansk, Poland
- 1962
- Pothast, K., Problems with smoking meat and meat products. Fleischwirtschaft, 55:1492
- 1975
- _____, Smoking methods and their effect on the content of 3,4 benzopyrene. Fleischwirtschaft, 1978 58:371
- Rhee, K. and L.J. Bratzler, Polycyclic hydrocarbon composition of wood smoke. J.Food Sci., 1968 33:626-31
- Rutz, J.K., M. Kopalava and K. Pelikanova, The influence of some factors on the contents of 3,4 benzopyrene in smoked pork back fat. Prun.Potravin, 22:106
- 1971
- Steinig, J., 3,4 Benzopyrene content of smoked fish depending on smoking procedure. Z.Lebensmitt. Unters.Forsch., 162(3):235
- 1976
- Tilgner, D.J. and H. Daun, Influence of cellulose on the presence of carcinogens in curing smoke. 1964 Prezen.Spozv., 18:303
- _____, Anti-oxidative and sensory properties of curing smoke obtained by three basic smoke generation methods. Lebensmitt.Wiss.Technol., 3:77
- 1970

Table 1

Levels of 3,4 benzo(a)pyrene content in smoked oil sardines under different conditions

Combustion temperature Variables	Experimental samples					
	Control 400-600°C	I 300-400°C	II 300-400°C	III 300-400°C	IV 300-400°C	V 300-400°C
Smoking temperature and duration	45 + 5°C for 3.5 h 70 + 5°C for 2.5 h	45 + 5°C for 3.5 h 70 + 5°C for 2.5 h	45 + 5°C for 3.5 h 70 + 5°C for 2.5 h	45 + 5°C for 3.5 h 70 + 5°C for 2.5 h	45 + 5°C for 3.5 h	45 + 5°C for 3.5 h
Drying temperature and duration	Nil	Nil	Nil	Nil	70 + 5°C for 2.5 h Electrical drying	Sun drying for 4 to 5 h
Air supply	Freely allowed through shutters	Freely allowed through shutters	Controlled by closing shutters	Controlled by closing shutters	Controlled by closing shutters	Controlled by closing shutters
Filter	Not used	Not used	Not used	Filter (0.5-1.0 mm)	Filter (0.5-1.0 mm)	Filter (0.5-1.0 mm)
Colour formation	Intense yellow	Intense yellow	Intense yellow	Golden yellow	Light yellow	Light yellow
Taste and acceptability	Bitter taste not acceptable	Less bitter not acceptable	Bitter not acceptable	Good taste acceptable	Good taste acceptable	Rancid taste acceptable
3,4 benzo(a)pyrene content per kg of smoked product	11.73 µg/kg	6.9 µg/kg	3.73 µg/kg	2.4 µg/kg	1.8 µg/kg	1.6 µg/kg

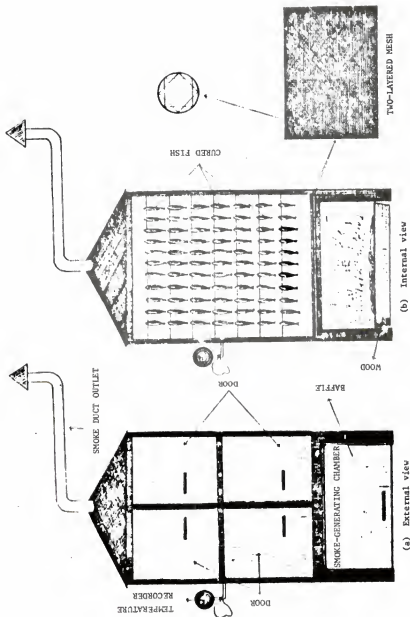


Figure 1 Smoke chamber

THE ROLE OF FAT CONTENT ON THE PROCESSING
OF BALI STRAIT SARDINES

by

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ABSTRACT

Oil sardine or temura (*Sardinella longiceps*) is one of the most important single species caught in the Bali strait and for fishermen of East Java and Bali it is their main source of living. The prospect for developing an oil sardine industry is bright because the oil sardine can be processed into salted, salted and boiled (pindang), canned fish, fish meal, marinated products, and silage as well as for bait for the longline fishery. However, effective utilization has been hampered by the biological characteristics of the fish, especially their high fat content. Investigations have been undertaken to predict variation of the fat content and its role on the suitability of the fish for specific processing methods and on the quality of the processed fish. Preliminary results show (a) the range of the fat content to be 5-25%; (b) there seems to be a correlation between the fat content and the fishing season; (c) possible utilization of the fish oil as the main product; and (d) better handling using crushed ice or iced seawater maintains the freshness of the raw material and reduces the detrimental effects of the high fat content. Additional results are presented and discussed.

1. INTRODUCTION

Fish are one of the most important sources of protein for human consumption in Indonesia. There is a potential for increasing fish landing and utilization, and fish is generally cheaper than meat. Oil sardines, locally known as temura are growing in economic importance in the Bali strait where more than 40 000 t are landed annually. The fishing season begins in April, reaching its peak in September/October. In 1983, approximately 83% of the catch from the western side of the Bali strait consisted of oil sardines.

During the peak of the fishing season oil sardines command a very low price as there is usually a glut in the market. Some of the problems faced by the industry are low quality raw material due to delays in chilling, high incidence of belly bursting because of actively feeding fish and the small size of the oil sardine (10-20 cm length) which can be easily damaged. However, if these problems are overcome there are good prospects to develop the industry. Fresh oil sardines are not generally consumed because of the high fat content and most of the catch is processed into different products.

1.1 Selting

Selting oil sardines is the oldest form of processing. In recent years the production of salted oil sardine has decreased. Selting is carried out in big cement tanks where the fresh fish are dumped into the tanks and salt added. The salting process is a combination of dry and brine selting where the brine produced during the selting process is used to process a new batch of oil sardines and dry salt added to maintain a certain degree of salt concentration. During periods of high fat content producers will obtain sardine oil as a by-product. After completion of the salting process (2-4 days), the fish is dried in the sun on bamboo mats and the oil is scooped from the surface of the brine.

1.2 Boiled-Selting Sardine

Boiled-selting fish or pindang is a popular processed fish in Java due to its low cost and agreeable taste. The present trend is to produce more pindang at the expense of salted fish. There are two slightly different pindang products. The first is salted pindang which is prepared by arranging fresh fish in a container (earthen pot or metal container) with alternate layers of fish and salt. When the container is full an additional layer of salt is added with a small amount of water. The pot is then heated for 2-6 h until the fish is cooked and dried; cooled, peeled and marketed. The shelf life is 2-6 weeks, depending on the amount of salt used and the cooking time.

The second is hrined pindang which is prepared by arranging fresh fish in one or two layers in a rectangular or round bamboo basket and occasionally while waiting to dip baskets in hot brine the fish is salted. A stack of the baskets is then dipped in hot brine for 15-25 min (the brine is kept at about 20-25°C and the brine kept near boiling temperature). After cooking, fish is cooled, packed and marketed. The shelf life is about 2-3 days.

1.3 Canning

There are about 5-6 active canneries on both sides of the Bali strait which process oil sardine in tomato sauce. Canned sardine is a popular product in Indonesia, so most of the canned fish are for the domestic market.

During the peak season excess catch and fish offal are used as raw material for fish meal but the high fat content and traditional processing methods will affect the quality of the final product. Improved methods of production are required to upgrade the quality of fish meal from oil sardines, especially methods of reducing fat content. Another possibility of processing oil sardines is by marinating. In South Sumatra fish meat is mixed and cooked with cassava flour and eaten in a sauce made from vinegar, sugar, water and spices.

1.4 Oil Sardines as Bait

A constant supply of bait is essential for the Indonesian long-line fishery. In the past Pacific saury (*Cololabis atrata*) was imported frozen from Japan but after testing different species of small pelagic fish (oil sardines, milkfish, scad and halfbeaks) oil sardines were chosen as bait. Utilization of frozen oil sardine as bait helps to stabilise the price of fresh oil sardine during the peak season and high quality requirement for bait has taught traditional fishermen better handling techniques.

1.5 Fat Content of the Oil Sardine

Oil sardine caught in Bali strait has a high fat content, especially during the second half of the year. During its life cycle the fat content varies from as low as 1% to higher than 25%. These figures are based on data collected between September 1981 to May 1984 when it was found that the more fat stored in the belly cavity, the higher the fat content of the flesh.

Work was also carried out to assess how the fat content varied with different biological characteristics. Results showed that the fat content was lower in larger fish and the closer fish were to spawning. Research planned for the future includes a study on how the fatty acid composition varies with season in order to maximize the utilisation of this species.

1.6 Sardine Oil, its Advantages and Disadvantages

Utilisation of oil sardine resources should be directed for human consumption especially in developing countries like Indonesia. As a commodity sardine oil could be promoted to a valuable product for human consumption and for industrial use.

1.7 Quality of the Oil Sardine as Raw Material

The main disadvantages in using oil sardines is that they spoil within 8-7 h at ambient temperature. Brown discolouration occurs within 3 h when stored without ice, especially when the fat content is at a maximum. The overall result is a lower price, however storage in chilled seawater or ice will prevent discolouration. Other problems with storage prior to processing are belly hurting and rancidity, where the peak of the fishing season coincides with the highest fat levels.

1.8 Quality of the Processed Oil Sardine

It is well known that rancidity occurs in fish meal made from fatty fish faster than in meal made from demersal species and the freshly cooked oil sardine taken during period of low fat tasted better than the fish taken in September.

At the fishing centre (Muncar, Banyuwangi, East Java) most of the catch is processed into several kinds of products and only a small part is consumed in fresh condition. It seems that the best way to utilize the oil sardine is to process it into a popular product like canned sardine in tomato sauce or pindang. Due to high fat content freshly cooked oil sardine gives a fatty taste and a quassy feeling and salted sardine will rapidly go rancid which lowers its value as a commodity. The pindang made from oil sardine has relatively lower quality compared with pindang made from scad due to inferior taste and softer texture. Contrary to the fact that high fat content acts as constraint, marinade processing needs fatty fish like oil sardines to produce the necessary delicate taste and with small adjustment to the composition of spices used this new product has been accepted by panalists and selected consumers.

1.9 Processing Suitability of Oil Sardine

Availability and quality raw material as well as the fat content will determine the suitable method of processing oil sardine. According to Tenikewa (1971) to achieve successful canning operation the level of fat should not exceed 15%, otherwise the pre-cooked sardine will be covered with a dark brown liquid. This has been observed during canning experiments using oil sardine with a fat content of more than 20% (Moeljento, 1978), so it is logical that the same processing method will produce different quality end-products if the level of fat content of the raw materials is different.

The variation in fat content and seasons is shown for two samples in Table 1. In order to process fish the initial fat content of the raw material should be known and if required special treatment should be applied to lower the fat content.

Table 1

Fat content and seasonal variation of oil sardines

Time	Fat content (%)	Length (cm)	Weight of gonad (mg)
May 1982	8.7	16.02	6 986.0 ^{a/}
September 1982	19.68	16.35	324.6

^{a/} Mature gonad

For the purpose of reducing the fat content three different groups of samples were taken in July and October 1983 and February 1984 with fat levels of 13.93% in July, 11.20% in October and 15.25% in February. The samples were then processed by five different methods (selting, pindeng, canning, fish-meal processing and ensiling) and residual fat content determined. From the data obtained the ability of each processing method to reduce the level of fat can be calculated (Table 2). Fish-meal processing is the method which reduced the highest percentage of fat in the fish.

For human consumption, pre-cooking during canning is not effective in reducing the fat content, while selting is a better method. In practice sardine oil is collected and sold for additional income.

Table 2

The ability to reduce fat content by different processing methods

Sampling date	(% fat reduced - dry basis ^{a/})				
	Processing methods				
	Selting	Brined-pindeng method	Canning	Fish meal	Ensiling
July 1983	5.88	3.02	4.61	25.45	26.01
October 1983	7.32	1.82	6.90	10.49	5.51
February 1984	5.34	7.01	0.74	20.46	15.38

^{a/} Average values

1.10 Sardine Oil as a Commodity

At present sardine oil is collected and sold as by-product and the canneries as well as fishermen get additional income. The fish processing industry could benefit by making the sardine oil a profitable business.

During the peak fishing season the price of fresh oil sardine usually drops to a low level of about US\$ 50 to US\$ 75 per ton. With a modest fat content of 15%, 150 kg of sardine oil can be obtained from one ton of fresh fish. A drum (200 litres) of sardine oil with simple refining can be sold at about US\$ 80 while refining of oil for export will increase the value to US\$ 200/drum. So instead of utilizing the oil sardines as a source of animal protein, during the fishing season it may be more valuable to extract the oil and utilize the dried cake as fish meal, thus reducing imports.

Fish oil which contains long chain fatty acids (C_{20} and C_{22}) with five or six double bonds is considered as health food in Japan. The beneficial effects of unsaturated fatty acids (EPA = eicosapentaenoic acid, $C_{20:5}$ and DHA = docosahexaenoic acid, $C_{22:6}$) according to Takeuchi (1984) and Suzuki (1984) check coagulation of blood thus preventing thrombosis and heart diseases. The Eskimos in Greenland eat lots of fish and marine animals rich in EPA, in Japan the fish which are rich in lipids are now used as an effective agent in preventing geriatric diseases.

Sen, Bhandary and Murthy (1974) found through their experiments with rats that sardine oil has possible hypocholesterolemic effects on human blood. So it would be interesting to know whether the oil of oil sardine caught in Bali strait could be explored as a source of EPA and DHA, if it is so the production of oil from the oil sardine will be justified from the points of economic and nutritive values.

2. REFERENCES

- Moeljanto, R., Frozen oil sardine as raw material for canning. J.Fish Technol.Res., 1:53-65
1978
- Sen, D.P., C.S. Bhandary and I.A.S. Murthy, Hypocholesterolemic effect of sardine oil (*Sardinella longiceps*). J.Food Sci.Technol., Mysore, 11(3):113-7
1974
- Suzuki, T., Increasing the use of sardine and mackerel for human consumption. Infofish Market.Dig.,
1984 (1/84):34-6
- Takeuchi, M., Fish as health food in Japan. Infofish Market.Dig., (1/84):26-8
1984
- Tanikawa, E., Marine products in Japan. Tokyo, Koseisha-Koseikaku Co., pp. 71-211
1971

SALTED AND PRESSED SARDINES

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ABSTRACT

Sardines can be processed by salting and pressing to prepare a cured product which is preserved at ambient tropical temperatures. Although the process has been used in Brazil it has not as yet been developed for use in other tropical countries. In the present study European sardines were used in order to examine the effects of various processing parameters on the quality, composition and storage life of salted and pressed sardines. A product with a storage life of up to three weeks was prepared with rancidity and loss of texture being the major factors limiting its storage life. The degree of dressing of the fish, pressure and length of pressing period made only limited differences to the keeping quality of the products. It was concluded that the method was suitable for the preparation of a product of moderate storage life but further modifications such as the use of preservatives or anti-oxidants may be needed if longer storage lives at ambient temperatures are required.

1. INTRODUCTION

As conventional food fish resources are becoming increasingly fully utilized there is a need for alternative fish resources to be used for human consumption to face the world's growing population. Small pelagic fish such as sardines are often underutilized with some 17 to 21 million tons per year being reduced to fish meal and oil compared with only 11 million tons being used for direct human consumption. There are also resources which are not presently utilized even for meal production. Hence there is considerable potential for increasing the supply of fish for human food by improving the handling, processing and distribution systems for the pelagic catch. The small size and high fat contents of the fish make handling and processing difficult and the highly seasonal nature of many pelagic fisheries has also severely restricted utilization. The problems which are encountered during the handling and processing of this resource have been recently reviewed (Disney, Wood and Poulter, 1982). A range of technical options were discussed and it was concluded that a single process was unlikely to be suitable for all situations; there was a need to consider the relative merits of conventional and novel high and low technology techniques under the prevailing circumstances.

A novel low technology process which involves the salting and pressing of sardines has been developed in Brazil. The product is similar to some traditionally cured fish in that preservation depends on the same principles of reducing water activity. This is a measure of the amount of water available for biochemical reactions and microbial growth. The subject has been reviewed in detail by Waterman (1976) but for the purposes of this paper it should be noted that high salt contents and low moisture contents decrease water activity and inhibit microbes. Preservation therefore depends on salt and moisture contents, as well as other factors such as levels of microbial contamination, fat contents, quality of the raw materials, etc.

The processing method uses unsophisticated, inexpensive technology making it suitable for small to medium-scale operations preparing products suitable for distribution to lower income group consumers. The absence of a sun-drying stage may make it particularly suitable for use in larger fishing centres where land is not available to conduct this technique, while the storage life may be longer than that of a salted undried product.

The aim of this study was to conduct preliminary investigations of the process to determine how the processing parameters affect the characteristics of the product. This background study will assist in future work when the suitability of the process can be assessed in specific practical situations.

2. METHODS

2.1 Fish Processing

Locally available frozen European sardines (*Sardina pilchardus*) were used throughout. The proximate composition of the flesh was as follows: moisture 68.6%; protein 18.0%; fat 9.2%; ash 1.8%; acid insoluble ash 0.8%. After thawing the fish were dressed in various ways before being selted in saturated brine for various lengths of time at ambient laboratory temperatures (approximately 16°-20°C) or at 30°C. Additional salt was added to ensure that the solutions remained saturated throughout the brining period. For some batches 0.1% (v/v) propionic or glacial acetic acids were added to the brine as preservatives.

Pressing was conducted using a steel screw press which exerted pressure on batches of fish held in a 160 mm diameter perforated cylinder. Approximately 1.5 kg fish batches (undressed weight) were used per experiment. "Low" pressure was exerted by tightening the screw until it was hand tight while additional "high" pressure was exerted by using a hydraulic jack in the base of the press. Fish was held under pressure for 4 to 24 h at ambient laboratory temperatures.

For storage trials the pressed fish was transferred to polythene bags which were sealed and stored at ambient laboratory temperatures. At weekly intervals the fish was removed from the bags for assessment of texture, appearance, and odour.

2.2 Analysis

At various stages 3 fish were taken at random from a batch for analysis. The fish were headed, gutted and tailed (if they were not already dressed in this way) before grinding and mixing. A pooled sample was analysed by the following methods.

2.3 Moisture Content

Samples were dried overnight in an oven at 105°C. Moisture content was calculated as the percent loss in weight of the moist sample.

2.4 Fat Content

This was determined using a Soxtec HT System (Tecator Ltd.) which operates on the same principles as the Soxhlet procedure.

2.5 Salt Content

Salt was determined using a Chlor-O-Counter Mk 2 (Marius Instrumenten) silver electrode titration method.

3. RESULTS

3.1 Dressing and Salting

The effect of various dressing procedures on the composition of brined fish is given in Table 1 (along with data on subsequent pressing experiments). The data indicated that, as expected, dressing did lead to increased salt penetration but the differences were not particularly large. The study was extended to brining undressed and dressed fish at simulated tropical temperature (30°C) and monitoring the quality of the fish. The observations made at ambient temperature and 30°C are shown in Table 2, which indicates that dressing had an important effect on the texture of the product particularly at the higher temperature. An additional study on the brining of whole fish for 7 days at 30°C was undertaken to see if whole sardines could be adequately brined at this temperature. The data, shown in Table 3, indicated that whole fish could probably be satisfactorily brined for 3 days at 30°C but due to excessive softening of the flesh longer brining periods would not be possible unless this species was gutted.

3.2 Pressing

Low pressure, short-time treatment was used to treat sardines selted and dressed in different ways as shown in Table 1. Pressing reduced the moisture contents to similar levels in the product irrespective of the dressing treatment used. More extreme pressing procedures were tested as shown in Table 4. High pressure, long-time pressing caused the sardines to break up but the other procedures appeared satisfactory.

Table 1

Composition of sardines after 7 days brining at ambient temperatures and prassing at low pressure for 4 hours

Composition (%)	Trial No.	Dressing method					
		Whole		Descaled		Headed and Guttet	
		Brined	Brined and prassad	Brined	Brined and pressed	Brined	Brined and pressed
Moisture	1	62.9	57.5	62.5	57.0	63.2	57.5
	2	57.7	53.5	60.4	58.2	56.3	53.2
	average	60.3	55.5	61.5	57.6	60.0	55.4
Fat	1	9.8	9.6	9.3	12.0	11.0	11.2
	2	10.4	10.4	12.0	7.4	15.4	12.6
	average	10.1	10.0	10.7	9.7	13.2	11.9
Salt	1	8.9	11.4	13.1	12.4	10.4	10.1
	2	10.1	11.1	11.0	12.4	11.4	11.8
	average	9.5	11.2	12.1	12.4	10.9	11.0

Table 2

Quality characteristics of sardines dressed in differant ways and brined for 7 days at ambient temperatures (about 16°-20°C) and 30°C

Dressing method	Characteristics of sardines	
	Brined at ambient	Brined at 30°C
Whole	Belly intact but soft, flesh firm, fishy odour	Belly burst, flesh very soft and readily breaks up, off odours
Descaled	Belly intact but soft, flesh quite firm, neutral odour	As above
Headed and Guttet	Flesh mostly firm, fishy odour	Fleshy mostly firm, slight off odour

Table 3

Composition and characteristics of whole sardines brined at 30°C

Time (days)	Moisture (%)	Fat (%)	Salt (%)	Remarks
0	68.6	9.2	0.8	Fresh
1	62.4	12.5	3.2	Belly area soft, flesh firm
2	59.4	13.8	5.0	Belly burst in most of the fish, flesh firm
3	59.2	10.4	8.7	Off-odours from brine, belly burst, flesh becoming soft
4	58.3	8.4	9.7	Off-odours from brine, belly burst, flesh starting to break up
5	58.1	11.7	8.6	As above
6	57.1	11.2	11.6	Off-odours, excessive belly burst, flesh very soft and breaking up
7	55.3	11.4	13.5	As above

Table 4

Composition and liquid loss of pressed brined sardines (whole sardines, 4 days brining at ambient laboratory temperatures)

	Short time (4 hours)		Long time (24 hours)	
	Low pressure	High pressure	Low pressure	High pressure
Moisture (%)	61.9	59.2	62.2	54.6
Fat (%)	11.4	9.7	8.3	8.5
Salt (%)	3.7	5.3	6.1	9.0
Liquid lost (as % of wt. of sardines)	31.4	32.7	36.7	43.7
Oil lost (as % by volume of liquid lost)	11.8	8.6	8.2	30.9

3.3 Storage

Some batches of fish dressed in different ways, brined for 7 days at ambient laboratory temperatures and pressed for 4 h at a low pressure were stored at ambient laboratory temperatures. The quality of the product was assessed as shown in Table 5. Trials were terminated after 3 weeks as the product was considered to be unacceptable after this time.

Table 5

Storage of salted and pressed sardine at ambient laboratory temperatures
(sardines brined 7 days at ambient laboratory temperatures,
pressed at low pressure for 4 hours)

Process	Quality of given lengths of storage (in weeks)		
	1	2	3
Whole	Flesh firm, belly soft, slightly rancid odour.	Oil leaking out of flesh, flesh slightly soft, belly soft, slightly rancid odour.	Very oily flesh surface, dark brown discolouration. Flesh and belly soft, rancid odour.
Desceled	Flesh oily and firm, belly soft, slightly rancid odour.	Oil leaking out of flesh, flesh slightly soft, slight off-odours.	Very oily flesh surface dark brown discolouration. Flesh and belly soft, no off odours.
Headed and Gutted	Flesh oily and firm, slightly rancid odour.	Oil leaking out of flesh, fairly firm flesh, rancid odour.	Very oily flesh surface, dark brown discolouration. Flesh soft, rancid odour.
Whole plus 0.1% propionic acid in brine	Flesh oily and firm, belly soft, slightly acidic and rancid odour.	Oil leaking out of flesh, fairly firm flesh, belly soft, rancid odour.	Very oily flesh surface, dark brown discolouration. Flesh and belly soft, rancid odour.
Whole plus 0.1% acetic acid in brine	As above	As above	As above

4. DISCUSSION

4.1 Dressing and Salting

The brining trials indicated that the maximum duration of the process was determined by the rate at which the fish flesh softened. The flesh must retain a reasonably firm texture to give the desired product which essentially consists of intact fish. Short brining periods may, however, be inadequate to give a product with a sufficiently high salt content to ensure preservation during distribution. Hence the fish may have to be gutted to slow the rate of softening in order that the fish can be brined for an extended period to give a product with a higher salt content and hence a longer storage life, but this must be re-examined when specific types of fish are considered for specific markets. Certainly the pressing trials indicated that moisture removal during the process was limited so that high salt contents would be the most important means of lowering the water activity of the product.

The excessive softening of the fish texture is almost certainly due to enzymic action. Digestive enzymes hydrolyse protein destroying the muscle structure and allowing nutrients to be leached into the brine. Although enzymic digestion can be prevented by cooking this will in itself alter the texture of the fish. A preliminary trial indicated that cooked fish readily broke up during pressing to give an unsatisfactory product (King, unpublished data). Removal of the gut tissue which has particularly active digestive enzymes inhibits enzymic digestion but ambient tropical temperatures increase the rate of enzymic reactions. The level of enzymic activity at a given temperature will vary from species to species, depending to some extent on the normal environmental temperatures in which the fish is found. As the European sardines inhabit waters which are generally cooler than those which tropical sardines species inhabit, the behaviour of these fish at ambient laboratory temperatures (about 16° to 20°C) may be more representative of the behaviour of tropical species under tropical conditions. Hence the problems of excessive softening of European sardines at 30°C do not necessarily indicate that all tropical species would need to be gutted if brining times of more than about 3 days are required; this would have to be investigated using the specific species at the ambient temperature.

Dressing appears to have a fairly minor effect apart from the way it affects softening. Due to the heterogeneity of the fish the increase in salt penetration resulting from descaling or heading and gutting was only just detectable by the experimental procedure used. The composition of the fish being processed will probably be as significant in this respect as the way in which it is dressed. Hence the length of brining required to give a product with a given storage life will probably be largely determined by the nature of the raw material. Particular markets may, of course, prefer fish which are dressed in a particular way but otherwise it can probably be kept to the minimum (i.e., undressed or headed and gutted if necessary).

4.2 Pressing

High pressure for an extended time was very effective at expressing moisture and oil, as a result, would give a product less susceptible to microbial attack and possibly also to rancidity. It was, however, unsatisfactory as the integrity and texture of the fish was damaged giving a product which would almost certainly be unacceptable to a potential consumer. Less drastic pressing procedures gave a block which contained fish which still retained their characteristic appearance and texture. The fish in the block tended to stick together after the application of even fairly low pressures aiding subsequent packaging. The effect on the composition of the fish was, however, not particularly great. The dressing procedure used seemed to have no significant effect on compositional changes caused by pressing.

4.3 Storage

Storage trials indicated that the major quality defects which developed were the separation of oil from muscle tissue, the oxidation of fat leading to the dark brown discolouration and off-odours associated with rancidity in cured fish and the excessive softening of the flesh. Neither dressing nor the addition of propionic or acetic acid made any great differences to the storage properties.

The oil separation and softening may well have been part of the same process, namely the breakdown of flesh. This was probably due to enzymic and possibly also microbial action although mould growth did not become apparent during these trials. Rancidity is caused by the oxidation of fish fat by atmospheric oxygen. The reduction of the surface area of the fish exposed to the air achieved by pressing it into larger blocks and the use of vapour-proof wrapping materials should reduce the rate of oxidation.

5. CONCLUSIONS

The products prepared were considered to be acceptable for three weeks when stored at ambient laboratory temperatures. This could possibly be extended by using longer brining periods to reduce the water activity of the fish even further before the pressing stage but heading and gutting may be necessary to ensure that the fish can be satisfactorily brined for long periods without softening. The effect of pressing on the water activity of the product is probably of secondary importance compared to the brining stage. The significance of pressing is most probably that it reduces the surface area of the fish by giving a cohesive block. This will in itself reduce the exposure of the fish to the atmosphere hence inhibiting rancidity. There may be scope for adding preservatives or anti-oxidants if these are effective and if the storage life of the product needs to be extended.

The process appears to be a promising method of obtaining an inexpensive product with a moderate storage life. Trials need to be continued using larger samples (some initial trials have started at TDRI) and the process should be assessed in suitable places (TDRI plans to collaborate with the College of Fisheries, Mangalore, India on this aspect).

6. REFERENCES

- Disney, J.C., C.D. Wood and R.G. Foulter, The use of small pelagic fish for human food. Paper presented at the meeting of the International Association of Fish Meal Manufacturers, Cannes, France, 1982 (mimeo)
- Waterman, J.J., The production of dried fish. *FAO Fish.Tech.Pap.*, (160):52 p. Issued also in 1976 French and Spanish

MOISTURE SORPTION ISOTHERMS OF DRIED-SALTED FISH

by

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ABSTRACT

Adsorption and desorption isotherms were carried out on dried-salted trevally (*Uca caranx nobilis*) and sardines (*Sardinella* spp.) at 10, 25, 35 and 50°C for salt content between 11-48% (dry basis). Water activity for the adsorption isotherms was determined by allowing the sample to equilibrate with selected saturated salt solutions of known relative humidities and for the desorption isotherms by using an electronic hygrometer. The effects of salt, temperature, hysteresis and fish species were investigated and an empirical model developed to fit the sorption isotherms. This model was tested satisfactorily, resulting in eight distinct regression equations for predicting adsorption and desorption isotherms of dried-salted fish.

1. INTRODUCTION

Salting and drying are widely used for preserving fish throughout the world. In Asia these preservation methods have been used for many centuries and the dried products are consumed as traditional staple food. Processing methods are simple. Fish are gutted, split and salted in saturated brine or mixed with dry salt before being laid out to sun dry. The drying techniques have been improved by using solar driers (Chakraborty, 1976; Deng *et al.*, 1979; Doe *et al.*, 1977; Ismail, 1983; Pablo, 1979; Trin and Curran, 1983).

The stability of salted and dried fish products depends on their water activity (A_w) which is a measure of the water available to support the growth of microorganisms (Scott, 1957). The relationship of A_w to moisture content is usually presented in the form of a sorption isotherm. Some sorption data for fish products have been published previously. Table 1 summarizes this work by listing the type of isotherms, type of products and temperature of the experiment.

Sorption isotherms of foods are normally described in terms of mathematical models, which can be either theoretical or empirically based (Henderson, 1952; Chirife and Iglesias, 1978; Van der Berg and Bruin, 1981). Boquet, Chirife and Iglesias (1978, 1979) tested eight two-parameter and four three-parameter equations with published sorption data for 39 foods and concluded that the water sorption data of probably any food would be represented satisfactorily by one of only four isotherm equations, namely Hailwood and Horrobin, Halsey, Oswin or Iglesias and Chirife.

The purpose of this study is to obtain sorption isotherms of dried-salted fish at refrigeration and three ambient temperatures. From the sorption data, empirical equations were developed which will be useful in designing drying processes and prediction of storage conditions of dried-salted fish.

2. MATERIALS AND METHODS

2.1 Fish Species

Trevally (*Uca caranx nobilis*) and sardine (*Sardinella* spp.) are the two fish species used in this study.

Trevally is a marine low-fat fish while sardine is a marine medium to high-fat fish. The fish samples were bought from the Fish Marketing Authority of NSW, Pyrmont, Sydney.

2.2 Processing Methods

Trevally fillets were sliced (about 5 mm thick) and sardine fillets were cut into small pieces to allow salt to penetrate quickly and reach equilibrium in a reasonable time. The samples from each fish were divided into three groups and then salted in 5, 15% and saturated brine respectively

for 24 h at 15.5°C. After salting was completed the fish were separated from brine and allowed to drain. Samples were taken from each group of salted fish for moisture, salt and fat determination.

The fish were subdivided into two halves. The first half was freeze-dried and stored in a desiccator and these samples were used in the adsorption experiments. The second half was air-dried in an experimental tunnel drier at 40°C with air velocity at about 2.5 m/sec. In order to obtain product with different water contents, samples were drawn from the drier at pre-programmed time intervals. The samples were individually ground and kept in airtight containers for several days at 4.4°C to equilibrate the water content distribution through the sample before being examined.

2.3 Water Activity Measurement

Water activity measurements for the desorption isotherms were determined with a Nova sine AG model enZFBA-3(4) ePP. Duplicate samples of air-dry salted fish were placed in plastic containers. Saturated salt solutions were also placed in similar containers. The samples and salt solutions were maintained at constant temperature for 24 h before measurements were taken. The meter was calibrated against saturated salt solutions of known A_w (Wexler and Hasegawa, 1954; Young, 1967). The meter was then used to estimate the A_w of the samples. The moisture contents were analysed in duplicate after the measurement of A_w .

For adsorption experiments, the freeze-dried samples were ground and weighed in plastic dishes. Duplicate samples (2 g) were placed over saturated solutions of various salts of A_w in the range 0.30-0.80 in plastic desiccators maintained at constant temperature. Weights were taken daily until constant weight was reached. When the samples reached constant weight within 2-4 weeks they were analysed in duplicate for moisture content.

2.4 Proximate Analysis

Moisture content in salted trevally as well as dried-salted trevally was determined by drying 2-5 g samples in a convection oven for 24 h at 105°C.

Moisture content in salted sardine as well as dried-salted sardine was determined by drying 2-5 g samples in a vacuum oven - 80 KPa for 40 h at 70°C.

Salt contents were measured using the silver nitrate method with potassium chromate as indicator (Pearson, 1970).

Fat contents were determined using the acid hydrolysis method (Wills and Greenfield, 1982). The fish samples are boiled with dilute hydrochloric acid to free bound lipids. The lipid, extracted with petroleum ether and diethyl ether, was determined gravimetrically after evaporation of the ethers.

3. RESULTS AND DISCUSSION

The results of the initial proximate analysis of three groups (salted in 5, 15% and saturated brine) of salted trevally and sardine are shown in Tables 2 and 3. Samples from the 2nd batch in Table 2 were used to obtain desorption isotherms of dried-salted trevally at 35 and 50°C because insufficient sample was obtained from the 1st batch. Samples from the 2nd batch in Table 3 were used to obtain desorption isotherms of dried-salted sardine.

The results of the desorption and adsorption isotherms for both fish types are shown in Table 4 to 7. The moisture contents on salt free, fat free dry basis (calculated from the results of the initial proximate analysis) are also given.

An attempt to fit the data to known theoretical relationships was made. Use of two-parameter equations, Cassie, Henderson and Halsey relationships (Boquet, Chirife and Iglesias, 1978) resulted in a poor correlation. Prediction was adequate only for the samples of low salt contents. A three-parameter equation was then applied to fit the data. According to Boquet, Chirife and Iglesias (1979) and Ferro Fontan *et al.* (1982), Railroad and Horrobin's equation was the best equation for fitting experimental data of almost any type of food. This equation has three parameters to be determined and was developed in an attempt to interpret the water sorption isotherms of proteins. The equation (a.q. 1) showed an excellent fit for both dried-salted fish isotherms in the A_w range 0.30-0.75.

$$M = A_w / (a + bA_w + cA_w^2) \quad (1)$$

where a , b and c are regression coefficients and M is moisture content expressed on percent dry basis. Coefficients for the isotherm equations are shown in Tables 8 and 9. The agreement between some experimental and calculated sorption isotherms obtained from the regressions in Tables 8 and 9 are shown in Figures 1 to 4. These equations were used to determine the significance of the differences between isotherms.

The effect of salt content on the sorption isotherms observed in this work is similar to that found by Cooper and Noel (1966) for salted cod. At constant A_w , the quantity of sorbed water increased with decreasing salt content. The adsorption data at 25°C were plotted on a salt free, fat free dry basis as shown in Figures 5 and 6. All values of equilibrium moisture content at A_w below 0.75 fall on the single curve while at A_w above 0.75 the equilibrium moisture content ($9w/Mb$) increased as salt content increased. This result agrees with those found by Cooper and Noel (1966) and Doe *et al.* (1982) for salted cod. The isotherms for each salt level were compared by the regression comparison method (Neter and Wasserman, 1974). The results showed that the differences in the isotherms due to changes in salt content were highly significant (Table 10).

Adsorption isotherms for both fish types at different temperatures showed that the quantity of sorbed water at a given A_w increased as temperature decreased (Figures 7 and 8). The isotherms at each temperature were compared by the method mentioned previously and the results showed no significant difference in isotherms in the range 25-35°C (Table 11).

The effects of sorption mode (adsorption and desorption) for both fish types are shown in Figures 9 and 10. Experimental points corresponding to adsorption and desorption fall over the same curve up to A_w values of approximately 0.65. Above this point, both fish types had a higher equilibrium moisture content in the desorption mode. The differences in moisture content indicate a hysteresis effect. The differences in isotherms due to changes in sorption mode are shown in Table 12. The results showed that the differences in isotherms were highly significant for the samples of low salt content.

The effect of fish species on the isotherms was found to be small. The differences in isotherms due to changes in fish species are shown in Table 13. The results showed that in most cases, the differences in isotherms were not significant.

Multiple linear regression techniques were used to develop a relationship which included the variables A_w , moisture content and salt content. It was found that the data from this work could be represented satisfactorily by an empirical equation of the following form:

$$\ln A_w / (0.76 - A_w) = b_0 + b_1 \ln M + b_2 S + b_3 \ln M S \quad (2)$$

Where A_w = Water activity

M = Moisture content (% db)

S = Salt content (% db)

b_i = Constants

This equation was fitted to the experimental data using a SPSS package and the results of this analysis are shown in Tables 14 and 15. The coefficient of correlation (R^2) for all regression lines indicates that the model was a satisfactory representation of the experimental data. The agreement between some experimental and calculated sorption isotherms from the regression equations in Tables 14 and 15 are shown in Figures 11 to 14.

Because fish species had such a small effect on sorption isotherms, this effect can be neglected. Table 16 presents the results of regression analysis carried out neglecting fish species and the coefficients of correlation (R^2) show that the experimental data still gave a good fit.

4. CONCLUSION

It may be concluded that the use of the Hailwood and Horrobin's equation to predict the sorption isotherms of dried-salted fish is adequate for the A_w range 0.30-0.75.

The empirical equation developed can describe the experimental data satisfactorily. The effect of salt content on the sorption isotherms of dried-salted fish is highly significant. Temperature in the range 25-35°C had no effect on sorption isotherms. The effect of sorption mode on sorption isotherms was significant only for low salt content and the effect of fish species was insignificant.

5. REFERENCES

- Boquet, R., J. Chirife and R.A. Iglesias, Equation for fitting water sorption isotherms of foods. 1978 2. Evaluation of various two-parameter models. *J.Food Technol.*, 13:319-28
- _____, Equations for fitting water sorption isotherms of foods. 3. Evaluation of various three-parameter models. *J.Food Technol.*, 14:527-34
- Chakraborty, P.K., Solar drier for drying fish and fishery products. *Res.Ind.India*, 21:192-4
- 1976

- Chirife, J. and H.A. Iglesias, Equations for fitting water sorption isotherms of foods. 1. A
1978 review. J.Food Technol., 13:159-74
- Cooper, D.L. and T.C. Noel, Some equilibrium moisture values of fresh and salted cod. J.Fish.Res.
1966 Board Can., 23(5):775-8
- Curren, C.A. and R.G. Poulter, Isohalic sorption isotherms. 3. Application to a dried-salted
1983 tropical fish *Xenomugil thoburni*. J.Food Technol., 18:739-46
- Deng, J.C., et al., Drying seafood products with solar energy. Changing energy use futures. In
1979 Conference Proceedings, edited by R.A. Pazzolare and C.B. Smith. Oxford, UK., Pergamon
Press Inc., Vol 4:1884-92
- Doe, P.E., et al., A polythene tent drier for improved sun drying of fish. Food Technol., Aust.,
1977 29:437-41
- _____, Isohalic sorption isotherms. 1. Determination for dried salted cod (*Gadus morhua*).
1982 J.Food Technol., 17:125-34
- Ferro Fontan, C., et al., Analysis of a model for water sorption phenomena in foods. J.Food Sci.,
1982 47:1590-4
- Henderson, S.M., A basic concept of equilibrium moisture. Agric.Eng., 33:29-32
1952
- Ismail, M.S., Solar driers for fish. Infofish Market.Dig., 2:31-3
1983
- Lupin, H.M., Principles of salting and drying hake. FAO Fish.Rep., (203)Suppl. 1:161-76. Issued
1978 also in Spanish
- Moschiar, S.M., J.P. Pardin and R.L. Boeri, Sorption isotherms of dried-salted hake (*Merluccius*
1984 *kubbi*). Lebensmitt.Wiss.Technol., 17:86-9
- Neter, J. and W. Wasserman, Topics in regression analysis. 1. In Applied linear statistical models.
1974 Homewood, Illinois, Richard D. Irwin, Inc., 160 p.
- Pablo, I.S., Solar drier for tropical fruits and marine products for rural development. NSDB
1979 Technol.J.Philipp., 1979 (Jan.-Mar.):26-41
- Pearson, D., The chemical analysis of foods. London, J. and A. Churchill, 6th. ed.
1970
- Scott, W.J., Water relations of food spoilage microorganisms. Adv.Food Res., 7:83
1957
- Trim, D.S. and C.A. Curren, A comparative study of solar and sun drying of fish in Ecuador. London,
1983 Tropical Products Institute
- Van der Berg, C. and S. Bruin, Water activity and its estimation in food systems. In Water
1981 activity: influences on food quality, edited by L.B. Rockland and G.F. Stewart. London,
Academic Press, pp. 1-63
- Wexler, A. and S. Hasegawa, Relative humidity-temperature relationships of some saturated salt
1954 solutions in the temperature range 0-50°C. J.Res.Natl.Bur.Stand.(Sect.A), 53:19-25
- Wills, R.N.H. and M. Greenfield, Laboratory instruction manual for food composition studies.
1982 Kensington, New South Wales, School of Food Technology, U.N.S.W.
- Young, J.F., Humidity control in the laboratory using salt solutions. J.Appl.Chem., 17:241-5
1967

Table 1

Published sorption data for fish

Author(s)	Isotherm	Products	Temperatures studied (degree C)
Cooper & Noel(1966)	Adsorption	Fresh and salted Cod fillet	20
Curran & Poulter(1983)	Adsorption	Dried salted Lisa	27
Cutting et al. (1956)	Adsorption	Freeze-dried raw Cod muscle	10
Cutting et al. (1956)	Adsorption	Air-dried cooked Cod muscle	15 & 37
Cutting et al. (1956)	Adsorption	Rolled-dried cooked Whiting muscle	10 & 37
Cutting et al. (1956)	Adsorption	Air-dried cooked Herring muscle	0,15 & 25
Doe et al. (1982)	Adsorption	Freeze-dried unsalted Cod	25
Lupin(1978)	Adsorption	Heavily salted Cod	...
Moachiar et al. (1984)	Adsorption & Desorption	Dried salted Hake	20,30 & 40

Table 2

Proximate analysis of salted trevally (% wet weight)

Group	1st Batch			2nd Batch		
	Moisture	Salt	Fat	moisture	Salt	Fat
1.Salted in 5% brine	81.48	3.74	1.86	82.10	3.41	2.21
2.Salted in 15% brine	76.63	9.46	1.99	76.66	10.11	1.75
3.Salted in saturated brine	68.68	14.77	1.73	67.71	15.71	1.78

Table 3

Proximate analysis of salted sardine (% wet weight)

Group	1st Batch			2nd Batch		
	Moisture	Salt	Fat	Moisture	Salt	Fat
1.Salted in 5% brine	70.28	3.26	9.06	76.76	3.62	4.06
2.Salted in 15% brine	61.63	7.82	13.64	69.48	10.30	4.40
3.Salted in saturated brine	55.07	13.62	11.68	61.15	15.21	6.06

Table 4

Desorption data for dried-salted trevally

10 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
106.34	1.52	0.87	99.18	1.94	0.75	80.89	1.71	0.75
57.57	0.82	0.78	38.41	0.75	0.74	33.66	0.71	0.75
29.99	0.43	0.69	18.59	0.36	0.69	15.11	0.32	0.70
15.79	0.23	0.59	13.56	0.26	0.66	8.16	0.17	0.57
11.23	0.16	0.51	6.82	0.13	0.43	5.30	0.11	0.37
8.84	0.13	0.39
25 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
104.28	1.49	0.87	98.89	1.94	0.75	80.52	1.70	0.75
57.59	0.82	0.78	62.24	1.22	0.74	55.25	1.17	0.75
29.13	0.42	0.69	38.56	0.76	0.73	32.90	0.70	0.74
14.86	0.21	0.59	17.77	0.35	0.69	15.77	0.33	0.70
10.76	0.15	0.49	13.59	0.27	0.66	7.98	0.17	0.59
8.45	0.12	0.40	6.97	0.14	0.49	5.46	0.12	0.43
35 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
93.93	1.37	0.84	92.81	1.89	0.75	90.38	1.97	0.75
39.89	0.58	0.73	31.42	0.64	0.73	56.99	1.24	0.75
25.90	0.38	0.70	13.60	0.28	0.69	28.57	0.62	0.73
13.88	0.20	0.62	5.73	0.12	0.50	4.89	0.11	0.47
8.79	0.13	0.48	4.27	0.09	0.38	4.38	0.10	0.39
6.60	0.10	0.37	3.76	0.08	0.33	3.89	0.08	0.36
50 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
94.49	1.38	0.85	94.02	1.91	0.75	90.22	1.97	0.74
40.33	0.59	0.72	33.68	0.68	0.73	55.91	1.22	0.74
26.00	0.38	0.70	13.82	0.28	0.68	28.95	0.63	0.73
13.54	0.20	0.61	11.68	0.24	0.66	4.61	0.10	0.50
8.13	0.12	0.46	5.73	0.12	0.50	4.11	0.09	0.42
6.37	0.09	0.36	4.78	0.10	0.43	3.76	0.08	0.40
...	3.77	0.08	0.33

Groups 1, 2 and 3 refer to Table 2

Mw = Moisture content (% dry basis)

Mw/Mb = Moisture content (salt free fat free dry basis)

Aw = Water activity

Table 5

Adsorption data for freeze-dried salted trevally

10 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
8.29	0.12	0.34	6.36	0.12	0.34	5.42	0.11	0.34
12.28	0.18	0.52	9.02	0.18	0.52	7.09	0.15	0.52
14.97	0.21	0.58	11.02	0.22	0.58	8.51	0.18	0.58
28.78	0.41	0.68	20.38	0.40	0.68	14.76	0.31	0.68
64.26	0.92	0.75	93.64	1.84	0.75	82.07	1.73	0.75
74.30	1.06	0.82	145.33	2.85	0.82	165.18	3.49	0.82
25 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
6.38	0.09	0.33	5.36	0.11	0.33	4.28	0.09	0.33
8.79	0.12	0.48	7.00	0.14	0.48	5.54	0.12	0.48
9.96	0.14	0.53	7.78	0.15	0.53	6.25	0.13	0.53
16.25	0.23	0.64	12.26	0.24	0.64	9.41	0.20	0.64
60.06	0.86	0.76	87.95	1.72	0.76	79.76	1.68	0.76
72.40	1.04	0.80	136.53	2.68	0.80	157.03	3.32	0.80
35 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
5.65	0.08	0.32	4.45	0.09	0.32	4.03	0.08	0.32
7.89	0.11	0.46	6.21	0.12	0.46	5.44	0.11	0.46
7.12	0.10	0.51	6.53	0.13	0.51	5.65	0.12	0.51
12.75	0.18	0.62	9.24	0.18	0.62	7.19	0.15	0.62
57.39	0.82	0.75	101.19	1.98	0.75	77.29	1.63	0.75
68.21	0.98	0.80	134.36	2.63	0.80	150.69	3.18	0.80
50 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
3.98	0.06	0.31	2.81	0.06	0.31	2.58	0.05	0.31
6.90	0.10	0.45	5.62	0.11	0.45	4.64	0.10	0.45
7.87	0.11	0.46	5.68	0.11	0.46	4.91	0.10	0.46
10.71	0.15	0.59	7.73	0.15	0.59	5.86	0.12	0.59
54.36	0.78	0.75	109.01	2.14	0.75	60.18	1.27	0.75
64.01	0.92	0.79	126.34	2.48	0.79	148.78	3.14	0.79

Groups 1, 2 and 3 refer to Table 2

Mw = Moisture content (% dry basis)

Mw/Mb = Moisture content (salt free fat free dry basis)

Aw = Water activity

Table 6

Desorption data for dried-salted sardine

10 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
46.00	0.69	0.75	54.31	1.05	0.75	82.36	1.82	0.76
12.69	0.19	0.55	47.44	0.92	0.75	61.39	1.36	0.76
9.79	0.15	0.43	12.87	0.25	0.66	28.76	0.64	0.74
8.58	0.13	0.37	8.50	0.16	0.52	19.59	0.43	0.72
5.86	0.09	0.22	5.43	0.10	0.28	10.43	0.23	0.64
...	4.48	0.09	0.23	4.56	0.10	0.31
25 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
45.28	0.68	0.74	53.13	1.02	0.75	80.70	1.78	0.75
12.21	0.18	0.56	46.05	0.89	0.75	61.47	1.36	0.75
9.59	0.14	0.46	12.43	0.24	0.66	27.64	0.61	0.74
8.46	0.13	0.38	8.42	0.16	0.54	18.86	0.42	0.72
5.92	0.09	0.25	5.10	0.10	0.30	9.75	0.22	0.65
...	4.24	0.08	0.25	4.71	0.10	0.34
35 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
43.35	0.65	0.72	51.01	0.98	0.74	80.30	1.77	0.74
11.66	0.17	0.55	44.94	0.87	0.74	61.30	1.35	0.74
9.15	0.14	0.47	11.95	0.23	0.66	26.18	0.58	0.73
7.50	0.11	0.37	7.60	0.15	0.54	17.90	0.40	0.71
5.44	0.08	0.24	4.67	0.09	0.28	8.80	0.19	0.63
...	4.00	0.08	0.24	4.14	0.09	0.29
50 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
40.26	0.60	0.72	49.38	0.95	0.74	77.38	1.71	0.74
11.01	0.16	0.56	42.80	0.82	0.74	59.45	1.31	0.74
8.05	0.12	0.47	10.46	0.20	0.64	24.24	0.54	0.73
6.89	0.10	0.38	6.34	0.12	0.53	17.05	0.38	0.71
5.16	0.08	0.26	4.11	0.08	0.32	8.03	0.18	0.64
...	3.80	0.07	0.27	3.81	0.08	0.33

Groups 1, 2 and 3 refer to Table 3

Mw = Moisture content (% dry basis)

Mw/Mb = Moisture content (salt free, fat free dry basis)

Aw = Water activity

Table 7

Adsorption data for freeze-dried salted sardine

10 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
7.71	0.13	0.34	6.41	0.14	0.34	5.94	0.14	0.34
9.34	0.16	0.52	8.14	0.18	0.52	7.50	0.17	0.52
11.13	0.19	0.58	9.38	0.21	0.58	8.53	0.20	0.58
16.56	0.28	0.68	14.01	0.32	0.68	13.11	0.30	0.68
33.67	0.58	0.75	44.57	1.01	0.75	39.38	0.90	0.75
37.06	0.63	0.82	86.50	1.96	0.82	102.06	2.34	0.82
25 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
6.25	0.11	0.33	5.00	0.11	0.33	4.42	0.10	0.33
7.54	0.13	0.48	6.36	0.14	0.48	5.69	0.13	0.48
8.11	0.14	0.53	6.92	0.16	0.53	6.54	0.15	0.53
12.21	0.21	0.64	9.56	0.22	0.64	9.40	0.22	0.64
32.95	0.56	0.76	53.94	1.22	0.76	54.67	1.25	0.76
37.20	0.64	0.80	85.86	1.95	0.80	102.36	2.34	0.80
37 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
5.11	0.09	0.32	4.11	0.09	0.32	3.84	0.09	0.32
6.32	0.11	0.46	5.38	0.12	0.46	5.01	0.11	0.46
7.07	0.12	0.50	6.03	0.14	0.50	5.39	0.12	0.50
10.29	0.18	0.62	8.75	0.20	0.62	8.16	0.19	0.62
30.01	0.51	0.75	37.82	0.86	0.75	34.57	0.79	0.75
35.50	0.61	0.80	78.75	1.79	0.80	93.30	2.14	0.80
50 degree C								
Group 1			Group 2			Group 3		
Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw	Mw	Mw/Mb	Aw
4.52	0.08	0.31	3.68	0.08	0.31	3.56	0.08	0.31
5.43	0.09	0.45	4.87	0.11	0.45	4.33	0.10	0.45
6.57	0.11	0.46	5.42	0.12	0.46	5.40	0.12	0.46
9.55	0.16	0.59	8.62	0.20	0.59	7.75	0.18	0.59
32.90	0.56	0.75	71.78	1.63	0.75	87.29	2.00	0.75
40.74	0.70	0.79	83.87	1.90	0.79	102.31	2.34	0.79

Groups 1, 2 and 3 refer to Table 3

Mw = Moisture content (X dry basis)

Mw/Mb = Moisture content (salt free, fat free dry basis)

Aw = Water activity

Table 8

Coefficients in equations for dried-salted trevally isotherms

Isotherm	a	b	c	R ²
Desorption:				
10 degree C 20.2% salt	0.0048	0.1981	-0.2422	0.987
10 degree C 40.5% salt	-0.2301	1.1668	-1.1280	0.994
10 degree C 47.2% salt	-0.1195	0.8279	-0.8592	0.973
25 degree C 20.2% salt	0.0589	0.0078	-0.0791	0.983
25 degree C 40.5% salt	-0.2986	1.4029	-1.3268	0.999
25 degree C 47.2% salt	-0.2193	1.2036	-1.1901	0.994
35 degree C 19.1% salt	0.0446	0.1005	-0.1749	0.976
35 degree C 43.3% salt	-0.0615	0.7127	-0.8165	0.989
35 degree C 48.6% salt	-0.0825	0.8020	-0.9012	0.997
50 degree C 19.1% salt	0.0588	0.0478	-0.1301	0.969
50 degree C 43.3% salt	-0.0600	0.7072	-0.8141	0.993
50 degree C 48.6% salt	-0.1794	1.2306	-1.3115	0.997
Adsorption:				
10 degree C 20.2% salt	-0.0240	0.3111	-0.3522	0.999
10 degree C 40.5% salt	-0.0652	0.5585	-0.6105	0.998
10 degree C 47.2% salt	-0.1185	0.8270	-0.8708	0.993
25 degree C 20.2% salt	-0.0230	0.3630	-0.4156	0.999
25 degree C 40.5% salt	-0.0696	0.6176	-0.6749	0.996
25 degree C 47.2% salt	-0.0952	0.8069	-0.8765	0.993
35 degree C 20.2% salt	-0.0569	0.5425	-0.5984	0.969
35 degree C 40.5% salt	-0.0738	0.6951	-0.7766	0.979
35 degree C 47.2% salt	-0.1162	0.9115	-0.9818	0.957
50 degree C 20.2% salt	0.0728	0.0778	-0.2047	0.976
50 degree C 40.5% salt	0.0916	0.1489	-0.3410	0.963
50 degree C 47.2% salt	0.0441	0.4020	-0.5809	0.939

general form: $M = Aw/(a + bAw + cAw^2)$ a, b & c = Constante R² = Coefficient of correlation

Table 9

Coefficients in equations for dried-salted sardine isotherms

Isotherm	a	b	c	R ²
Desorption:				
10 degree C 15.6% salt	0.0045	0.1994	-0.2435	0.987
10 degree C 33.8% salt	-0.0283	0.4459	-0.5134	0.964
10 degree C 39.2% salt	-0.1080	0.8485	-0.9070	0.993
25 degree C 15.6% salt	-0.0027	0.2483	-0.2989	0.980
25 degree C 33.8% salt	-0.0320	0.4876	-0.5622	0.974
25 degree C 39.2% salt	-0.2126	1.2805	-1.3033	0.987
35 degree C 15.6% salt	-0.0054	0.2866	-0.3541	0.991
35 degree C 33.8% salt	-0.0487	0.5988	-0.6877	0.976
35 degree C 39.2% salt	-0.1564	1.1337	-1.2187	0.984
50 degree C 15.6% salt	-0.0123	0.3458	-0.4208	0.993
50 degree C 33.8% salt	-0.0642	0.7130	-0.8154	0.997
50 degree C 39.2% salt	-0.2440	1.5258	-1.5888	0.991
Adsorption:				
10 degree C 11.0% salt	-0.0701	0.5098	-0.5138	0.996
10 degree C 20.4% salt	-0.1000	0.6854	-0.7001	0.978
10 degree C 30.3% salt	-0.1100	0.7484	-0.7627	0.983
25 degree C 11.0% salt	-0.0630	0.5312	-0.5494	0.998
25 degree C 20.4% salt	-0.0962	0.7437	-0.7823	0.981
25 degree C 30.3% salt	-0.0838	0.7402	-0.8009	0.991
37 degree C 11.0% salt	-0.0447	0.5131	-0.5592	0.998
37 degree C 20.4% salt	-0.0528	0.6310	-0.7092	0.993
37 degree C 30.3% salt	-0.0651	0.7142	-0.7953	0.996
50 degree C 11.0% salt	-0.0182	0.4422	-0.5173	0.980
50 degree C 20.4% salt	-0.0218	0.5522	-0.6787	0.997
50 degree C 30.3% salt	-0.0469	0.6816	-0.8090	0.984

general form: $M = Aw/(a + bAw + cAw^2)$ a, b & c = Constants R² = Coefficient of correlation

Table 10

Isotherm differences due to variation in salt content

Dried-salted trevally			
Temperature	Comparisons	Sorption mode	Fcal
10 degree C	20.2% vs 40.5% salt	D	30.55**
25 degree C	20.2% vs 40.5% salt	D	92.01**
25 degree C	40.5% vs 47.2% salt	D	15.31**
35 degree C	19.1% vs 43.3% salt	D	29.55**
50 degree C	19.1% vs 43.3% salt	D	44.90**
10 degree C	40.5% vs 47.2% salt	A	22.73**
25 degree C	20.2% vs 40.5% salt	A	50.44**
25 degree C	40.5% vs 47.2% salt	A	21.83**
Dried-salted sardine			
Temperature	Comparisons	Sorption mode	Fcal
10 degree C	15.6% vs 33.8% salt	D	12.43**
25 degree C	15.6% vs 33.8% salt	D	16.24**
25 degree C	33.8% vs 39.2% salt	D	7.06*
35 degree C	15.6% vs 33.8% salt	D	21.37**
50 degree C	15.6% vs 33.8% salt	D	117.11**
10 degree C	11.0% vs 20.4% salt	A	6.61*
25 degree C	11.0% vs 20.4% salt	A	9.39*
17 degree C	11.0% vs 20.4% salt	A	21.93**
50 degree C	11.0% vs 20.4% salt	A	10.46*

D = Desorption mode A = Adsorption mode

Fcal= Calculated F values

* 95% Significance level

** 99% Significance level

Table 11

Isotherm differences due to variation in temperature

Dried-salted trevally			
Salt content	Comparisons	Sorption mode	Fcal
40.5%	10 vs 25 degree C	D	1.50
43.3%	35 vs 50 degree C	D	0.05
40.5%	10 vs 25 degree C	A	17.04**
40.5%	25 vs 35 degree C	A	2.17
40.5%	35 vs 50 degree C	A	3.59
40.5%	25 vs 50 degree C	A	8.05*
Dried-salted sardine			
Salt content	Comparisons	Sorption mode	Fcal
33.8%	10 vs 25 degree C	D	0.42
33.8%	25 vs 35 degree C	D	0.80
33.8%	35 vs 50 degree C	D	2.23
33.8%	25 vs 50 degree C	D	7.86*
20.4%	10 vs 25 degree C	A	4.66
20.4%	25 vs 37 degree C	A	3.04
20.4%	37 vs 50 degree C	A	5.55*
20.4%	25 vs 50 degree C	A	9.53*

D = Desorption mode A = Adsorption mode

Fcal= Calculated F values

* 95% Significance level

** 99% Significance level

Table 12

Isotherm differences due to variation in sorption mode

Temperature	Comparisons	Sorption mode	Treatment	Fcal
25 degree C	Trevally vs Sardine	D	salted in 5% brine	3.92
25 degree C	Trevally vs Sardine	D	salted in 15% brine	4.42
25 degree C	Trevally vs Sardine	D	salted in saturated	4.68*
25 degree C	Trevally vs Sardine	A	salted in 5% brine	126.99**
25 degree C	Trevally vs Sardine	A	salted in 15% brine	4.46
25 degree C	Trevally vs Sardine	A	salted in saturated	0.56

D = Desorption mode A = Adsorption mode

Fcal = Calculated F values

* 95% Significance level

** 99% Significance level

Table 13

Isotherm differences due to variation in fish species

Dried-salted trevally				
Temperature	Comparisons	Salt content	Fcal	
25 degree C	Desorption vs Adsorption	20.2%	17.28**	
25 degree C	Desorption vs Adsorption	40.5%	13.04**	
25 degree C	Desorption vs Adsorption	47.2%	3.71	
Dried-salted sardine				
Temperature	Comparisons	Salt content	Fcal	
25 degree C	Desorption vs Adsorption	15.6% & 11.0%	49.19**	
25 degree C	Desorption vs Adsorption	33.8% & 20.4%	2.10	
25 degree C	Desorption vs Adsorption	39.2% & 30.3%	3.30	

Fcal = Calculated F values

* 95% Significance level

** 99% Significance level

Table 14

Multiple regression equations for isotherm of dried-salted trevally $\ln Aw/(0.76-Aw) = b_0 + b_1 \ln M + b_2 S + b_3 \ln M S$

Isotherm	b0	b1	b2	b3	R ²
10 degree C Desorption	-2.7741	1.1016	0.0011	0.0150	0.942
25 degree C Desorption	-3.9458	1.5513	0.0514	-0.0035	0.977
35 degree C Desorption	-3.9118	1.8490	0.0394	-0.0069	0.984
50 degree C Desorption	-3.9767	1.8405	0.0495	-0.0101	0.984
10 degree C Adsorption	-6.7317	2.6803	0.0898	-0.0231	0.980
25 degree C Adsorption	-7.4103	3.2972	0.0927	-0.0272	0.992
35 degree C Adsorption	-5.2361	2.6043	0.0645	-0.0211	0.981
50 degree C Adsorption	-3.7879	1.9698	0.0503	-0.0155	0.975

b1 = Regression coefficients

R² = Coefficient of correlation

Table 15

Multiple regression equations for isotherm of dried-salted sardine $\ln Aw/(0.76-Aw) = b_0 + b_1 \ln M + b_2 S + b_3 \ln M S$

Isotherm	b0	b1	b2	b3	R ²
10 degree C Desorption	-5.8171	2.3864	0.0421	0.0004	0.977
25 degree C Desorption	-6.1272	2.5693	0.0921	-0.0216	0.973
35 degree C Desorption	-4.8049	2.0572	0.0701	-0.0154	0.946
50 degree C Desorption	-4.5621	2.0319	0.0750	-0.014	0.952
10 degree C Adsorption	-6.8629	3.2042	0.0989	-0.0306	0.982
25 degree C Adsorption	-7.7654	3.9288	0.1427	-0.0538	0.987
37 degree C Adsorption	-5.7081	3.1140	0.0808	-0.0268	0.994
50 degree C Adsorption	-3.9839	2.3665	0.0798	-0.0374	0.981

b_i = Regression coefficients

R² = Coefficient of correlation

Table 16

Multiple regression equations for isotherm of dried-salted fish $\ln Aw/(0.76-Aw) = b_0 + b_1 \ln M + b_2 S + b_3 \ln M S$

Isotherm	b0	b1	b2	b3	R ²
10 degree C Desorption	-6.9365	2.8538	0.0894	-0.0213	0.925
25 degree C Desorption	-5.9732	2.4969	0.0907	-0.0226	0.960
35 degree C Desorption	-4.3609	1.9593	0.0532	-0.0111	0.963
50 degree C Desorption	-4.2775	1.9908	0.0618	-0.0156	0.958
10 degree C Adsorption	-5.7493	2.7859	0.0705	-0.0260	0.918
25 degree C Adsorption	-6.3137	3.3078	0.0726	-0.0288	0.965
35 degree C Adsorption	-5.1474	2.9376	0.0648	-0.0288	0.957
50 degree C Adsorption	-3.2206	1.9200	0.0399	-0.0158	0.966

b_i = Regression coefficients

R² = Coefficient of correlation

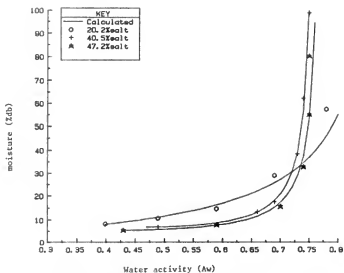


Figure 1 Experimental and calculated desorption isotherms of dried-salted trevally at 25°C

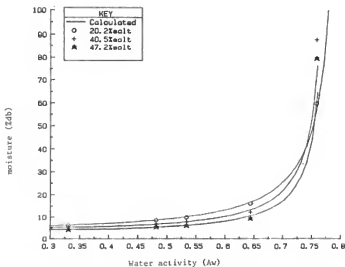


Figure 2 Experimental and calculated adsorption isotherms of dried-salted trevally at 25°C

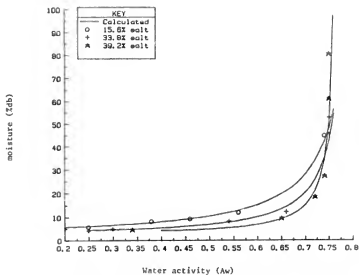


Figure 3 Experimental and calculated desorption isotherms of dried-salted sardine at 25°C

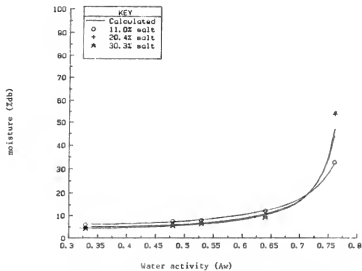


Figure 4 Experimental and calculated adsorption isotherms of dried-salted sardine at 25°C

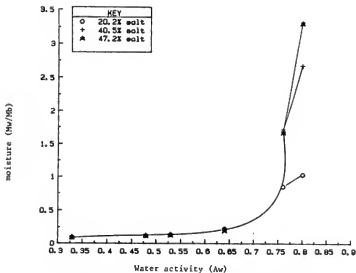


Figure 5 Adsorption isotherm of dried-salted trevally at 25°C (salt free, fat free dry basis)

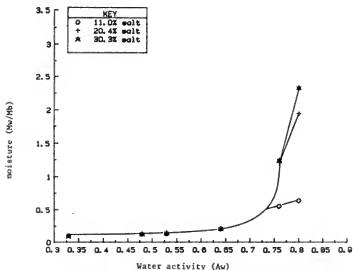


Figure 6 Adsorption isotherm of dried-salted sardine at 25°C (salt free, fat free dry basis)

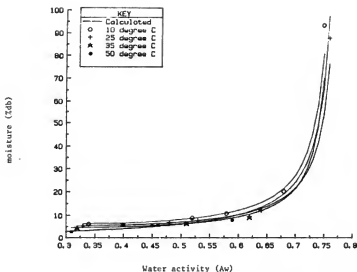


Figure 7 Effect of temperature on adsorption isotherm of dried-salted trevally (40.5% salt)

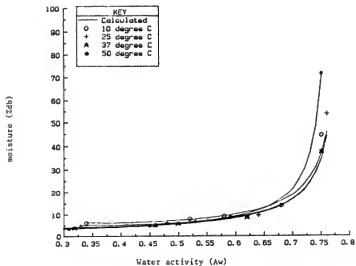


Figure 8 Effect of temperature on adsorption isotherm of dried-salted sardine (20.4% salt)

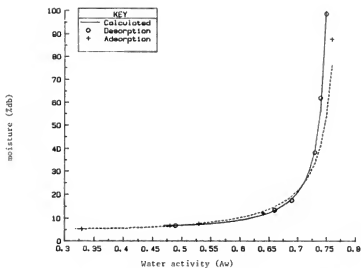


Figure 9 Effect of sorption mode on sorption isotherm of dried-salted trevally (25°C, 40.5% salt)

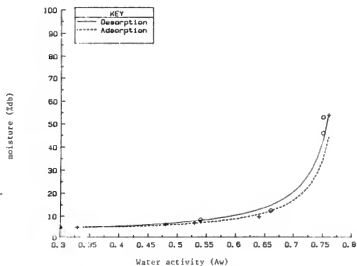


Figure 10 Effect of sorption mode on sorption isotherm of dried-salted sardine (25°C, salted in 15% brine)

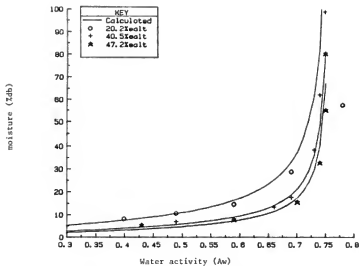


Figure 11 Experimental and calculated desorption isotherms of dried-salted trevally at 25°C

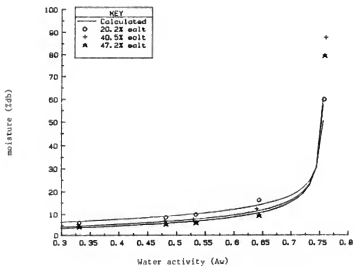


Figure 12 Experimental and calculated adsorption isotherms of dried-salted trevally at 25°C

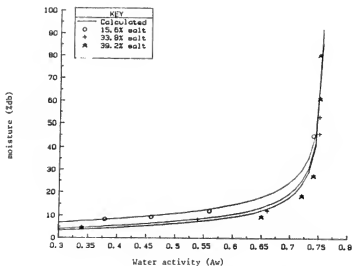


Figure 13 Experimental and calculated desorption isotherms of dried-salted sardine at 25°C

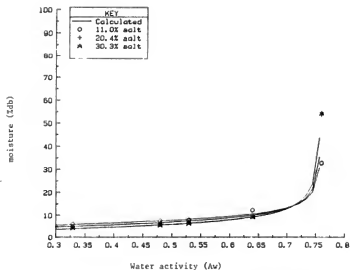


Figure 14 Experimental and calculated adsorption isotherms of dried-salted sardine at 25°C

INSECT INFESTATION IN DRIED-SALTED FISH IN JAVA

by

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ABSTRACT

Dried-salted fish occupies about 33% of the total catch disposition in Indonesia. In commercial practice dried-salted fish are not properly packed, and consequently become susceptible to insect infestation, especially during storage in the warehouse as well as during retailing. Studies showed that dried-salted fish with salt content of 7-21%, A_w 0.75 and stored under tropical climatic conditions (temperature between 28°C and 32°C, and RH of 73-87%) were heavily infested by insects, especially if they were packed in bamboo baskets or wooden boxes. The most common insects occurring on and contributing to heavy losses on dried-salted fish were flies, especially *Musca* sp., *Chrysomya* sp. and *Protophila* sp. Their maggots were commonly encountered in most of the dried-salted fish samples. In addition, *Dermeestes* sp., particularly *D. carnevorius* Fabricius, *D. maculatus* Degeer and *Neorobia rufipes* Degeer were frequently found in dried-salted fish. As for dried-salted freshwater fishes, in addition to the above-mentioned insects, mite (*Acarina*) infestations were observed. The basic life history and ecology of these insect pests are being documented with the objective of seeking non-chemical means for their control and improving fish quality; a method to rapidly assess levels of infestation in containers of fish scheduled.

1. INTRODUCTION

Indonesia, in common with many countries of tropical and subtropical regions, faces problems of high population and food supply. Significant losses occur in the food required to adequately feed the population through insect and mould infestation, and this is particularly relevant to fish protein which is a major component of the Indonesian diet. In addition to direct losses there is the possible involvement of spoilage organisms and other microbiota vectored by insect affecting human health.

During the period 1974-81, fishery production has increased from 1 336 268 t in 1974 to 1 408 272 t in 1981. About 33% of the catch was processed into dried-salted fish (Indonesia, Ministry of Agriculture, 1983).

Dried-salted fish are often processed by simple sundrying by artisanal fishermen. During sundrying, conditions are conducive to the development of Dipterian infestation. Larvae and pupae of various flies are common in fish specimens at drying sites (Blatchford, 1962).

After sundrying, the fish moisture content and salinity vary. Such conditions favour the oviposition, incubation and larval development of insects. Traditionally dried and salted fish are transported to the market place in woven bamboo baskets with dimensions of 16 cm high and 50 cm in diameter. Numbers of fish per basket vary with size and can range from 421 to 18 pieces for mackerel and catfish, respectively.

In November 1983 an investigation was commenced which aimed to establish a method by which the incidence of pest infestation within baskets of fish could be rapidly assessed without emptying the entire basket which is time-consuming and inconvenient to the owner.

2. MATERIALS AND METHODS

A total of 18 baskets have been completely examined to date. Individual fish in all layers were examined and the frequency and type of infestation recorded. The pests encountered were collected and identified by using "Common Insect Pest of Stored Food Product", (British Museum, 1980). The temperature and RH of the sites were also recorded.

3. RESULTS AND DISCUSSION

The major pests encountered were two species of flies, cheese skipper (*Piophilina casei*) (21%) and the large headed blowfly (*Chrysomya megacephala*) (1.3%), an undetermined number of *Dermestes* spp. (2.1%), mites (3.5%) and moulds (16.5%). The incidence of these pests is shown in Figure 1 and Table 1. Maggots of *Musca domestica* (Linn) were also observed at low frequency.

The relationship of percent infestation in the top four layers of any basket to the percentage total infestation, irrespective of the number of layers, promises to provide a relatively suitable method requiring about 10-15 min compared to 40 min to assess the entire content.

The regression of the relationship is shown in Figure 2. As shown, 93% of the variation in total infestation (r^2) was explained by variation in the estimates obtained from the top four layers and this was improved to 96% following log-log transformation of both variables. The respective correlation coefficients (r) were 0.97 and 0.98.

Whole fish such as *Rastrelliger* sp. and *Dussuriera* sp. in general were highly infested by larvae of *Piophilina casei* in the gills and mouth chamber. On the other hand, fatty fish, *Pangasius* sp., were attacked by larvae of *Chrysomya* sp. as well as *Dermestes* sp.

The dried unsalted fish, such as anchovy (*Stolephorus* sp.), were commonly infested relatively high by *Dermestes* sp. and mites.

Fish specimens after drying had water activities ranging from 0.70 to 0.75 and the salinity content ranged from 7% to 21%. These A_w were within the ranges found to be most favourable to the infestation of dried fish by mould, especially by the *Aspergillus* sp. (green mould) and *Penicillium* sp. (white mould).

Moreover, the ambient temperature ranged from 28° to 32°C and humidity (RH) from 73% to 87%. Such conditions which prevail during the storage and marketing of tropical commodities approximate to the optimal requirements of many stored products for insect and mould infestation.

4. CONCLUSION

Results to date showed that there is a relationship of percent infestation in the top four layers of any basket to the percentage total infestation irrespective of the number of layers. This relationship provided a relatively suitable method to assess the percentage total infestation in baskets in the continuing studies.

Sampling is being continued to validate the method and to gain a realistic appreciation of infestation levels in west Java and in time central and east Java.

At the same time, a series of sample questionnaires are being distributed to fish handlers throughout the entire marketing process to establish people's concepts of the presence of insect and mould infestation in relation to quality of fish. To date it is apparent that concepts of quality vary extremely.

5. REFERENCES

- Blatchford, S.M., Insect infestation problems with dried fish. Trop.Stored Prod. Inf., (4) 1962
- British Museum, Common pest of stored products. Brit.Mus.Econ.Ser., (15) 1980
- Indonesia, Ministry of Agriculture, Directorate General of Fisheries, Fisheries statistics of 1983 Indonesia. No. 11. Fish.Stat.Indones., (11)

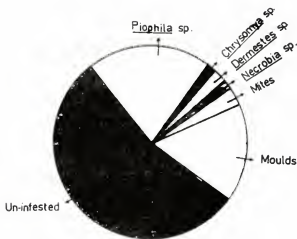


Figure 1

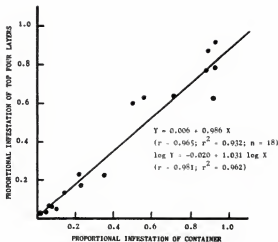


Figure 2

Table 1

Insect and mould infestation of dried-salted fish

Basket No.	Fish species	Form/size	Storage period (day)	Temp/°C	FISH KILL CHRYSIDAE			DERMES		NECROBIA		MITE		MOULD	
					SP.	VIA SP.	SP.	SP.	SP.	SP.	SP.	SP.	SP.	SP.	SP.
1	<u>Rastrelliger</u> sp. whole (chub mackerel)	fish ±16 cm	14	32/73	88.59	-	-	-	-	-	-	-	-	-	-
2	<u>Hemirhamphus</u> sp. (half beak)	splitting ±15 cm	14	32/73	0.94	-	-	-	-	-	-	-	-	47.17	-
3	<u>Rastrelliger</u> sp. whole (chub mackerel)	±15 cm	7	31/85	18.18	-	-	-	-	-	-	-	-	0.82	-
4	<u>Pangasius</u> sp. splitting (catfish)	±41 cm	3	31/85	22.22	-	-	-	-	11.11	-	-	-	-	-
5	<u>Dussumieria</u> sp. whole (fringe-scale sardines)	±19 cm	30	31/85	-	-	-	-	-	1.15	-	-	-	81.61	-
6	<u>Puntius javanicus</u> (puntius)	splitting ±21 cm	5	29.5/84	7.61	1.08	-	-	-	-	-	-	-	25.0	-
7	<u>Mugil</u> sp. (mullet)	splitting ±16 cm	7	29.5/84	1.82	2.87	-	-	-	-	-	-	-	-	-
8	<u>Dasyatis</u> sp. (rays)	splitting ±46 cm	7	29.5/84	0.37	2.21	-	-	-	-	-	-	-	-	-
9	<u>Ophiocephalus</u> sp. (snake head)	splitting ±18 cm	14	28.5/84	-	2.87	2.87	-	-	-	-	-	-	-	-

Table 1 (continued)

Basket No.	Fish species	Form/size	Storage period (day)	Temp/RH [°C/(%)]	(%) Pest			
					Plophila sp.	Chrysom- yid sp.	Dermes- tes sp.	Macrobia Mites Mould sp.
10	<u>Mugil cephalus</u> (mullet)	splitting 14 cm	7	28.5/84	3.89	-	-	-
11	<u>Puntius javani-</u> <u>cus (punctus)</u>	splitting 14 cm	45	30/78	22.07	-	0.27	0.54 - 88.55
12	<u>Ophiocephalus</u> <u>sp. (snake head)</u>	splitting 21 cm	14	29/77	1.19	0.59	0.59	4.76 73.81 -
13	<u>Selaroides</u> sp.	splitting 16 cm	7	28.5/85	0.56	-	-	1.13 -
14	<u>Dussumeria</u> sp. (fringe-scale sardines)	whole 23 cm	5	28/85	-	-	-	- 2.32
15	<u>Decapterus</u> sp. (scad)	whole 18 cm	7	29.5/87	74.68	2.53	-	- -
16	<u>Dussumeria</u> sp. (fringe-scale sardines)	whole 23 cm	7	29.5/87	91.89	-	-	- -
17	<u>Mugil cephalus</u> (mullet)	splitting 20 cm	7	29.5/87	5.88	5.88	-	- 13.72
18	<u>Pseudosciaena</u> sp. (croaker)	splitting 24 cm	14	29.5/87	-	8.69	-	- 73.91

SALTING AND DRYING OF *Nemadactylus macropterus* FILLETS
IN THE LABORATORY

by

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ABSTRACT

The effects of brining time and drying temperature on the quality of *Nemadactylus* sp. filets were examined. Salt uptake was essentially complete after eight hours in saturated brine and the quality of the dried product was impaired by brining for more than 24 h. Drying temperatures of 60°C and above gave a brittle product of burnt appearance. The dried product had an acceptable storage life of 16 days at 30°C, 60% relative humidity.

1. INTRODUCTION

The early part of the research project was designed to study the effect of salting and drying parameters on product quality. Conditions were chosen to simulate those commonly experienced in the tropics. Brining in saturated salt solution was chosen as a method of salting as it is commonly used in Southeast Asia. *Nemadactylus macropterus* was selected for this study since its size and shape resembled that of Mergui, a popular Malaysian dried-salted fish product and is readily available from the Sydney market. This species is captured mainly in the eastern Indian and Southwest Pacific Ocean regions.

2. MATERIALS AND METHODS

N. macropterus is commonly known as morwong, jackass morwong or deep seabream. They were bought from the fresh market and scaled, cleaned and filleted prior to salting on the same day.

Moisture was determined by oven-drying method of AOAC (1973) and salt by the Volhard method, and A_w was measured on the Novasine humidity meter calibrated using saturated solutions of appropriate salts.

2.1 Brining

Fillets were immersed in saturated brine for periods up to 72 h at 30°C. Samples were taken for analysis and drying at suitable intervals. They were rinsed with tap water for 5 sec and excess moisture wiped off. Samples for analysis were immediately homogenized in a blender and stored at -18°C until analysed. Samples for drying were kept overnight at 5°C in sealed polyethylene bags prior to further treatment. The fillets were laid skin side down on perforated aluminium trays and dried at various temperatures to a moisture content of approximately 30-40% in a custom-built tray drier which allowed change in weight during drying to be followed.

An airflow of 2.5 m/sec and a loading of 0.9-3.2 kg of fillets were used in all experiments. Temperature and relative humidity of the incoming air were measured using a wet and dry bulb thermometer.

3. RESULTS

Table 1 shows the salt and moisture contents at various times during salting of morwong fillets together with A_w , salt and moisture contents of fillets dried for 24 h at 50°C, 45% RH after each brining time.

Examination of data for the fillets before drying showed that salt uptake reached its maximum after 8 h brining with little subsequent change, while moisture loss, quite rapid up to 24 h almost ceased during longer brining.

Drying of fillets brined up to 24 h yield products of approximately 36% moisture content while salt levels reflected those found in the undried fillets. The dried product of 72 h brining had the highest moisture content of 49.1%. Fillets brined for longer than 24 h gave dried products

which displayed a number of physical defects. The upper surfaces were brittle with visible salt crystals. Longer brining may have denatured the protein leading to decreased water-holding capacity. This would allow diffusion of moisture to the surface of the fish in the early stages of drying leading to crystallization of salt as drying progressed. High surface salt levels may in turn cause the brittleness. The fillets brined for 72 h also showed evidence of surface brittleness and salt crystals, but underneath the flesh was soft and moist. This extended drying time may have initiated the formation of a layer of poor moisture permeability which restricted water loss during drying resulting in the high moisture content of this product.

Reconstitution of the dried fillets gave satisfactory products for brining times up to 24 h, although some tendency of the flesh to break into segments was apparent. Fillets brined for longer than 24 h gave dried products which not only had a greater propensity for fragmentation but their surface regions tended to disperse as small particles. It appears that the extent of brining can also affect fragmentation.

3.1 Drying

Eight hours brining was adopted for the investigation of drying conditions. Figure 1 shows losses in weight with time during drying at various temperatures and relative humidity combinations. Drying rate, obviously faster at higher temperatures, is also affected by RH. For example, at 30°C and 55% RH, moisture content dropped to 45.9% after 28 h, while in another experiment at 30°C and 40% RH the product reached similar moisture levels after only 13 h. In the absence of control, RH was determined by drying temperature and ambient conditions. However, variation in RH at a given temperature had no apparent effect on product quality.

Several defects became obvious in the dried product as higher temperatures were used. *Morwong*, dried at 60°C and 70°C, had a burnt appearance, was very brittle and reconstituted poorly to give fragmented pieces of meat blocks. They reconstituted very slowly as compared to the others giving poor texture that was dry, rather tough and rubbery.

The best product was that dried at 30°C which had desirable pale colour and was pliable. On reconstitution it had good appearance and texture and did not fragment. Satisfactory products were obtained using drying temperatures up to 50°C. Although drying at 30°C gave the best quality in the absence of RH control, 50°C appears to be the most compromise between quality and drying time for tropical areas. Air at 30°C and 90% RH on heating to 50°C should decrease to approximately 30% RH and dry the product to below 40% moisture content in about 30 h.

4. CONCLUSION

We would conclude by saying that it is possible to prepare salted-dried fish product from *Nemadactylus macropterus*. However, it should be noted that these conditions have been established for *morwong* fillets alone and are not necessarily of general applicability. Factors such as fish size and shape and fillets or whole fish and the presence of skin will affect drying rate.

Currently, we are looking at the effects of these processing conditions on the protein in the fish electrophoretically and also on the ultrastructure of the fish microscopically. In the future we will be looking at the salting and drying conditions of a few other species, their digestibility and also storage behaviour.

5. REFERENCES

AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1975 AOAC

Table 1
Fish species bought at retail markets

Fish species			
<u>Scientific name</u>	<u>Indonesian</u>	<u>English</u>	<u>Number tested</u>
<u>Rastrelliger sp.</u>	Kembung	Mackerel (chub)	9
<u>Auxis thazard</u>	Tongkol	Mackerel (frigate)	6
<u>Scomberomorus sp.</u>	Tengiri	Mackerel (spanish)	8
<u>Chanos chanos</u>	Bandeng	Milkfish	10
<u>Mugil cephalus</u>	Belanak	Mullet	8
<u>Formia niger</u>	Bawal hitam	Pomfret (black)	10
<u>Katsuwonus pelamis</u>	Cakalang	Skipjack tuna	5
<u>Lutjanus sanguineus</u>	Ikan merah	Snapper	9
<u>Selaroides leptolepis</u>	Selar kuning	Trevally (yellow stripe)	8

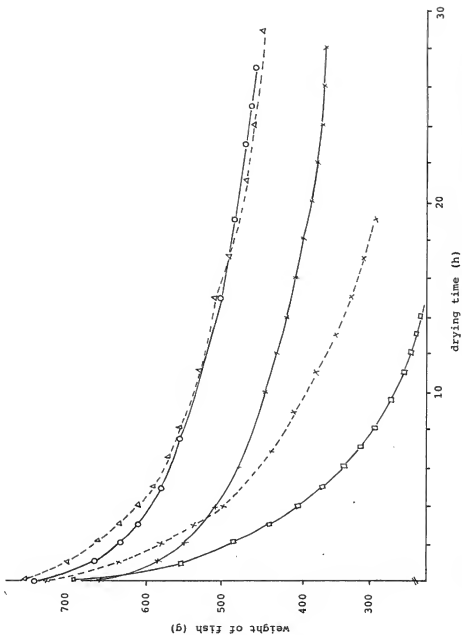


Figure 1 Drying of 8-h salted fillets at various temperatures in ambient relative humidities

WATER ACTIVITY DATA IN RELATION TO QUALITY LOSS FOR
SOUTHEAST ASIAN CURED FISH

by

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ABSTRACT

The water activities of a wide range of salted/dried fish and smoked fish products from Southeast Asia are reported and discussed in relation to mould, aflatoxin and histamine formation. For two samples of salted/dried marine catfish (*Arius* sp.) and two samples of salted/dried red snapper (*Lutjanus* sp.), water activity data are given for samples of flesh taken from different parts of the fish. Changes in water activities during salting, drying and initial storage of marine catfish and Spanish mackerel (*Scomberomorus commersonii*) are also reported.

1. INTRODUCTION

About 15% of the world fish catch is preserved by curing, i.e., salting, drying or smoking, or a combination of these treatments. Fish curing is of particular importance in Southeast Asia where it accounts for almost 30% of the fish catch. However, a considerable proportion of cured fish produced in that region does not reach the consumer because of "spoilage" during processing and distribution, or is of poor quality when it reaches the consumer. Mould formation is an important cause of spoilage of cured fish (FAO, 1981). Another aspect of quality loss for cured fish is the development of toxic compounds. Two toxic compounds which have been reported in cured fish are the carcinogen aflatoxin B₁ (Shank, *et al.*, 1972; Okookwo and Mwakolo, 1978) and histamine, which is implicated in an allergic-like response known as scombretoxin poisoning (Taylor, 1983). Aflatoxin B₁ is produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Histamine formation in fish flesh is thought to result mainly from the action of bacteria possessing the enzyme histidine decarboxylase. Large amounts of histidine are naturally present in pelagic fish flesh.

Water availability, which can be usefully expressed as water activity (a_w) is an important factor in the formation of mould, aflatoxins and histamine. Visible mould growth occurs on cured fish after about 4 days at $a_w = 0.85$, 10 days at $a_w = 0.80$, 40 days at $a_w = 0.75$ and 100 days at $a_w = 0.70$ (Poulter, Doe and Olley, 1982). Aflatoxins are not produced at water activities below about 0.85 (Moss, 1984). Histamine-forming bacteria are unlikely to be viable at water activities below about 0.90, although extra-cellular bacterial enzymes would still be active.

Very little a_w data have been reported for Southeast Asian cured fish products and no data for changes in a_w during processing and storage.

2. MATERIALS AND METHODS

2.1 Materials

Cured fish products were obtained from processors, wholesalers and retailers in Burma, Indonesia, Malaysia, Philippines and Thailand. The samples were divided into 6 groups (Table 1) with salted/dried fish over 20 cm in length (excluding the caudal fin) being placed in the "large salted/dried fish" group.

2.2 Methods

Water activities were determined in triplicate at $25 \pm 0.1^\circ\text{C}$ using a Novasina water activity meter.

Moisture was determined by drying samples to constant weight at $105 \pm 2^\circ\text{C}$. Protein was determined by the Kjeldahl procedure using a Kjeltac Auto 1030 Analyser; the data given in the tables are "crude protein" calculated using a protein factor of 6.25. Lipid was determined by a modified Bligh and Dyer procedure, except for samples F3 and F4 when the Soxhlet procedure was used. Ash was determined at 500°C and NaCl in the ash samples by silver citrate titration using potassium chromate

as the indicator. Mean values of triplicate determinations are given in the tables, with the following approximate errors: moisture ± 1 , protein ± 1 , lipid ± 0.5 , ash ± 1 , NaCl ± 1 .

3. RESULTS AND DISCUSSION

Table 1 summarizes the a_w data for 112 samples of cured fish^{1/}. The data were obtained on representative samples (about 10 g, in triplicate) taken after homogenizing about 1-2 kg of the cured fish products (i.e., a single fish for some large fish products or several fish for small fish products). Less than 5% of the samples had water activities that would permit the formation of aflatoxins or allow histamine-forming bacteria to survive, however, 11% had water activities of 0.80 or above, which would permit visible mould formation within 10 days. The types of products with the highest water activities were the large salted/dried fish, which are preferred in a soft, moist condition by many consumers in Southeast Asia, and the smoked fish, which in some cases were smoked for flavour rather than for preservation.

Little information is available on variation in water within large cured fish samples, although it is possible that within a sample with a relatively low average water activity, there are areas of high water activity, where spoilage processes might occur more readily. To obtain information on variations in water activities within samples, two samples of salted/dried marine catfish (*Arius* sp., each about 1 kg in weight) were sampled at three different positions, as shown in Table 2. Although the thick fleshy parts (labelled A and C) had higher water activities than the thin parts (labelled B), mainly as a result of the latter having higher NaCl contents, the difference was only about 0.03 water activity units. Two samples of salted/dried red snapper (*Lutjanus* sp., each about 1 kg in weight), were also sampled at various positions as shown in Table 3. For one fish the water activities differed by only 0.01 water activity units, in the other case by 0.05 water activity units with the tail section having the lowest values. Very little difference was observed outer and inner portions of fish flesh.

It is possible that mould, aflatoxin B, or histamine might develop during processing or the initial stages of storage when water activities are relatively high. Very little information is available on changes in water activity during processing of cured fish and in the period immediately following processing. To obtain such data for salted/dried fish, samples of a large lean fish, marine catfish (*Arius* sp., -1.1 kg gutted weight), and a large fatty fish, Spanish mackerel (*Scomberomorus commersoni*, -1.3 kg gutted weight), were processed in West Java by a traditional pickle-salting, sun-drying procedure (during the dry season). Samples were taken for water activity determination after salting, after each day's drying (3 days for the catfish, 2 days for the mackerel) and after 7 days storage. The results are given in Figures 1 and 2 in the Appendix.

For the catfish, only during the third day's drying does the surface a_w of the thick flesh portion fall below the limit for aflatoxin production (0.85). Whether significant amounts of aflatoxin could accumulate during drying needs to be further investigated. At the commencement of storage, the surface a_w of the fleshy part is still at 0.80, i.e., visible mould formation could occur within about 10 days.

For the mackerel, the water activities for the thick bony side are significantly higher than for the thin side. Being a pelagic fish, high histamine levels could build up in the flesh of the bony side since the a_w of the internal portion is above 0.90 until the 2nd day of drying. The surface a_w of the bony side is above 0.85 at the commencement of storage, raising the possibility of mould formation within a few days. In general the results suggest that the thick bony side of fish would be far more susceptible to mould formation than the thin side. This was often found to be the case; the thick sides of the fish after a week's storage were often completely covered with mould whilst the thin sides had only a few mould spots or were completely free from mould.

4. CONCLUSIONS

- (1) Large salted/dried fish and smoked fish were found in general to have higher water activities than other cured fish products from Southeast Asia and many of these products would be susceptible to visible mould formation within a few days.
- (2) For four large salted/dried fish samples, differences in water activities between different parts of the fish were not greater than 0.05 water activity units.
- (3) During the production of large salted/dried fish, differences in water activities of up to 0.1 water activity units were found between different parts of the fish and consequent differences in the susceptibility to mould formation were observed.

^{1/} Proximate analysis data were obtained for 47 of these samples; these are given in the Appendix

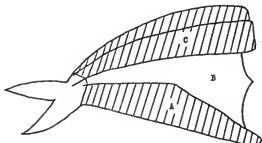
Table 1

Water activity data for cured fish from Southeast Asia

Type of cured fish (number of samples)	a_w		Σ with $a_w > 0.80$
	Range	Mean	
Large salted/dried fish (36)	0.67-0.92	0.75	17
Salted/dried anchovy (21)	0.66-0.76	0.72	0
Other small salted/dried fish (32)	0.55-0.85	0.72	3
Salted/dried shrimp (6)	0.62-0.76	0.70	0
Salted/dried squid (4)	0.53-0.72	0.63	0
Smoked fish (13)	0.56-0.96	0.74	38
All samples	0.53-0.96	0.72	11

Table 2

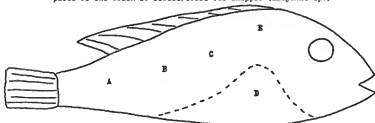
Water activity and other analytical data for samples taken from different parts of the flesh of salted/dried marine catfish (*Arilus* sp.)



Sample code	Percentage wet weight basis					Water activity
	moisture	protein	lipid	ash	NaCl	
F9/A	44	42	2.7	12.8	10.6	0.78
F9/B	40	40	3.3	16.6	14.1	0.74
F9/C	45	40	2.2	12.4	11.0	0.77
F10/A	47	42	2.3	13.4	11.5	0.81
F10/B	46	38	2.3	15.4	13.5	0.78
F10/C	45	42	1.9	12.4	12.2	0.81

Table 3

Water activity and other analytical data for samples taken from different parts of the flash of salted/dried red snapper (*Lutjanus* sp.)



Sample a/ code	Percentage wet weight basis					Water activity
	moisture	protein	lipid	ash	NaCl	
F3/Ao	49	34	0.1	19.2	18.5	0.74
F3/Ai	50	32	0.2		18.7	0.74
F3/Bo	49	34	0.4		17.2	0.74
F3/Bi	51	32	0.1		17.8	0.75
F3/Co	49	34	0.2		16.0	0.75
F3/Ci	51	31	0.1		16.2	0.75
F3/Dr	41	41	0.3		18.8	0.75
F3/Di	46	37	0.2		17.3	0.75
F3/Eo	51	32	0.4	15.0	17.4	0.74
F3/Ei	53	29	0.3		16.5	0.74
F4/Ao	50	31	2.5		15.3	0.77
F4/Ai	52	30	1.6		15.8	0.77
F4/Bo	53	31	2.0		14.8	0.79
F4/Bi	56	30	0.8		14.9	0.81
F4/Co	56	26	2.0		13.9	0.82
F4/Ci	56	27	0.4		14.7	0.80
F4/Dr	52	34	0.5		13.7	0.80
F4/Di	54	31	1.2		14.5	0.80
F4/Eo	53	31	0.8		14.2	0.80
F4/Em	56	28	0.1		15.3	0.81
F4/Ei	57	28	0.2		15.1	0.81

a/o = outer section of flash, beneath the skin; i = inner section of flash, around the backbone; m = middle section of flash between the outer and the inner sections (only for F4/E); r = flesh from right belly flap; l = flesh from left belly flap.

4. REFERENCES

- FAO: The prevention of losses in cured fish. FAO Fish.Tech.Pep., (219):87 p. Issued also in French 1981
- Moss, M., Review of studies on the microbiology of mycotoxin formation with particular reference to the conditions affecting toxin production during storage. Paper presented at the Conference on 'Mycotoxins' organised by the Society of Chemical Industries, London 1984
- Okonkwo, P.O. and C. Nwokolo, Aflatoxin B₁: simple procedures to reduce levels in tropical foods. 1978 Nutr.Rep.Int., 17:387-95
- Poulter, R.G., P.E. Doe, and J. Olley, Isohalic sorption isotherms. 2. Use in the prediction of storage life of dried fish. J.Food Technol., 17:201-10 1982
- Shank, R.C., et al., Dietary aflatoxins and human liver cancer. 3. Aflatoxins in market foods and foodstuffs of Thailand and Hong Kong. Food Cosmet.Toxicol., 10:61-9 1972
- Taylor, S.L., Monograph on histamine poisoning. Codex Alimentarius Commission. Rome, FAO/WHO, 1983 CX/FH 83/11:75 p.

APPENDIX

Table 1a

Large salted/dried fish - sample details

Sample code	Country of origin	Common name	Species or genus	Product
F1	Thailand	queenfish	<u>Chorinemus</u> sp.	whole
F2	Burma	yellow croaker	<u>Protonibea</u> sp.	eviscerated
F3	Malaysia	red snapper	<u>Lutjanus</u> sp.	eviscerated
F4	Malaysia	red snapper	<u>Lutjanus</u> sp.	eviscerated
F5	Burma	threadfin	<u>Eleutheronema tetradactylum</u>	fillet
F6	Burma	threadfin	<u>Eleutheronema tetradactylum</u>	fillet
F7	Indonesia	Spanish mackerel	<u>Scomberomorus commersonii</u>	split
F8	Indonesia	sea catfish	<u>Arius</u> sp.	beheaded
F9	Indonesia	sea catfish	<u>Arius</u> sp.	and
F10	Indonesia	sea catfish	<u>Arius</u> sp.	split
F11	Indonesia	tuna		eviscerated
F12	Indonesia	sting ray		wing

Table 1b

Large salted/dried fish - analytical data

Sample code	Percentage wet weight basis					Water activity
	moisture	protein	lipid	ash	NaCl	
F1	60	27	3.4	12.3	6.6	0.92
F2	34	45	2.6	21.0	15.8	0.74
F3	49	34	0.2	19.2	17.4	0.74-0.75
F4	54	30	1.7	15.0	14.7	0.77-0.82
F5	41	37	1.4	18.7	15.6	0.70
F6	40	43	3.8	13.7	11.0	0.69
F7	44	35	9.7	14.6	11.0	0.81
F8	36	41	5.0	17.8	11.9	0.73
F9	43	41	2.7	13.9	12.0	0.74-0.78
F10	46	41	2.1	13.7	12.4	0.78-0.81
F11	43	34	3.3	18.9	13.2	0.74
F12	44	31	1.2	24.5	21.4	0.73

Notes: The data were obtained on the whole cured fish products except for samples F3, F4, F9 and F10 where the data were calculated from the values given in Tables 2 and 3, which are for the fish flesh only.

Table 2

Salted/dried anchovies (*Stolephorus* spp.) -
sample details and analytical data

Sample Code	Country of origin	Percentage wet weight basis					Water activity
		Moisture	protein	lipid	ash	NaCl	
F13	Thailand	23	57	5.4	16.2	8.7	0.70
F14	Malaysia	27	52	5.8	16.1	10.1	0.72
F15	Malaysia	28	51	7.4	14.3	8.3	0.74
F16	Indonesia	18	63	5.8	11.9	3.4	0.70
F17	Indonesia	35	28	2.9	34.7	30.0	0.71
F18	Philippines	15	66	5.1	13.5	1.6	0.66

Table 3a

Other small salted/dried fish - sample details

Sample Code	Country of origin	Common name	Species or genus	Product
F19	Thailand	Freshwater sardines		eviscerated
F20	Thailand	silver grunt	Pomadasys sp.	beheaded split
F21	Burma	Bombay duck	Harpodon nehereus	split
F22	Malaysia	blue-barbed whiptail	Pentapodus sp.	split
F23	Malaysia	blue-barbed whiptail	Pentapodus sp.	split
F24	Indonesia	tamban	Sardinella sp.	whole
F25	Indonesia	Bombay duck	Harpodon nehereus	whole
F26	Indonesia	Bombay duck	Harpodon nehereus	fragments
F27	Indonesia	beunte		whole
F28	Indonesia	sepat	Trichogaster sp.	whole
F29	Philippines	lapu-lapu	Epinephelus sp.	split
F30	Philippines	labahita	Acanthurus sp.	beheaded split
F31	Philippines	tamban	Sardinella sp.	whole

Table 3b

Other small salted/dried fish - analytical data

Sample code	Percentage wet weight basis					Water activity
	moisture	protein	lipid	ash	NaCl	
F19	39	34	2.9	20.2	11.0	0.78
F20	39	37	2.1	21.0	16.1	0.75
F21	13	53	12.6	21.6	9.1	0.55
F22	25	34	3.5	34.1	21.3	0.74
F23	35	35	1.1	30.2	18.6	0.74
F24	41	35	3.4	20.1	12.5	0.76
F25	30	37	2.4	26.7	22.0	0.70
F26	27	29	2.7	38.6	28.1	0.72
F27	39	27	10.0	19.6	13.0	0.76
F28	28	32	10.7	22.2	9.5	0.73
F29	11	48	5.6	33.7	18.4	0.59
F30	26	44	3.5	26.0	18.3	0.68
F31	15	52	9.7	25.0	18.8	0.61

Table 4

Salted/dried shrimp and salted/dried squid - sample details and analytical data

Sample code	Country of origin	Type of shellfish	Percentage wet weight basis					Water activity
			moisture	protein	lipid	ash	NaCl	
F32	Thailand	shrimp	34	39	1.3	24.4	15.5	0.73
F33	Burma	shrimp	20	65	1.8	16.0	11.2	0.64
F34	Thailand	squid	16	67	3.3	7.9	3.9	0.55

Table 5a

Smoked fish - sample details

Sample code	Country of origin	Common name	Species or genus	Product
F35	Thailand	freshwater catfish	<u>Kryptopterus</u> <u>sp.</u>	eviscerated
F36	Thailand	lizard fish	<u>Saurida</u> <u>sp.</u>	split
F36	Thailand	lizard fish	<u>Saurida</u> <u>sp.</u>	beheaded split
F38	Thailand	snakehead	<u>Channa</u> <u>sp.</u>	split
F39	Thailand	lizard fish	<u>Saurida</u> <u>sp.</u>	beheaded split
F40	Thailand	lizard fish	<u>Saurida</u> <u>sp.</u>	beheaded split
F41	Thailand	freshwater catfish	<u>Kryptopterus</u> <u>sp.</u>	eviscerated
F42	Burma	freshwater catfish		eviscerated
F43	Malaysia	tuna		fillet
F44	Philippines	milkfish	<u>Chanos</u> <u>chanos</u>	eviscerated
F45	Philippines	milkfish	<u>Chanos</u> <u>chanos</u>	eviscerated resmoked
F46	Philippines	scad	<u>Decapterus</u> <u>sp.</u>	whole
F47	Philippines	scad	<u>Decapterus</u> <u>sp.</u>	whole

Table 5b

Smoked fish - analytical data

Sample code	Percentage wet weight basis					Water activity
	Moisture	protein	lipid	ash	NaCl	
F35	9	55	26.7	11.6	0.2	0.61
F36	12	71	4.4	15.2	1.3	0.68
F37	15	58	2.7	25.1	12.0	0.66
F38	16	61	10.2	15.0	9.5	0.65
F39	16	67	10.3	11.1	3.5	0.68
F40	27	55	7.5	13.6	6.4	0.80
F41	16	57	21.3	10.2	0.2	0.72
F42	8	68	6.9	14.9	0.1	0.56
F43	21	70	3.7	3.9	<0.1	0.85
F44	54	26	13.6	7.0	3.2	0.96
F45	28	41	14.4	14.0	7.5	0.77
F46	53	28	8.5	10.8	6.5	0.92
F47	39	40	7.5	14.2	9.6	0.81

Sampling positions: ● thick fleshy part
○ thin part

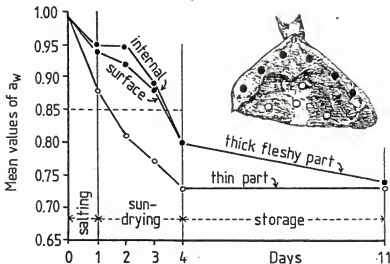


Figure 1 Changes in a_w during processing and storage of marine catfish (~1.1 kg gutted weight)

Sampling positions: ● thick bony side ○ thin side

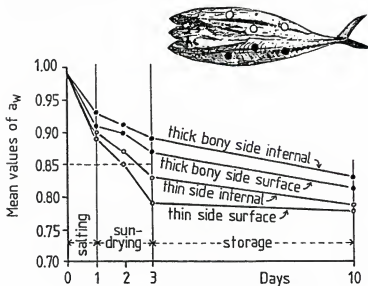


Figure 2 Changes in a_w during processing and storage of Spanish mackerel (~1.3 kg gutted weight)

LOSS REDUCTION TECHNIQUES FOR SALTED/DRIED MARINE CATFISH
(*Arilus* sp.)

by

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ABSTRACT

Various techniques were investigated in West Java for reducing losses of *Arilus* sp. during salting and drying. Increasing the salting time from one to two days did not significantly reduce blowfly infestation (the main cause of losses) during drying and was not acceptable to the processor. Covering the brining tank with a solid screen was found to be effective in preventing blowfly infestation during salting and this method was readily adopted by the processor. Screening of the drying tables significantly reduced blowfly infestation during drying, but was found to be inconvenient and impractical and was not acceptable to the processor. A newly developed pyrethroid insecticide, Fastac, (1Rcis)S and (1Scis)R alpha-cyano-3-phenoxybenzyl 3-(2, 2-dichlorovinyl)-2, 2-dimethyl-cyclopropanecarboxylate, was found to be effective in preventing losses during both the salting and drying stages. This insecticide had never previously been used with fish. The effectiveness of dipping spraying of Fastac at various concentrations (0.05% to 0.001%) was investigated. Dipping fish before drying at concentrations as low as 0.001% proved effective in reducing losses. Although residue levels are not yet available, the concentrations of Fastac used are lower than has previously been reported for any other effective insecticide treatment of drying fish. The Fastac-dipping technique was readily adopted by the processor and should be acceptable to other processors, particularly those who at present use household insecticides such as Baygon and Startox to reduce blowfly infestation.

1. INTRODUCTION

Surveys and experimental work carried out by the authors in West Java, Indonesia, over the past three years have identified blowfly infestation as being the most important cause of losses to salted/dried fish during processing and the early stages of storage. Large, low-salt, high-moisture products such as ikan jambal (salted/dried *Arilus* sp.) are particularly susceptible to blowfly damage and, in desperation, processors have resorted to the use of general household insecticides such as Baygon (dichlorvos with propoxur) and Startox to control the problem. This practice demonstrates an urgent need for the development and implementation of safe, effective techniques for the reduction of blowfly infestation and consequent losses.

2. *Arilus* PROCESSING METHODS

The fish, which is generally iced but of variable freshness, is purchased at the local landing site and transported by "becak" (tricycle-taxi) to the processors' premises and usually processed immediately upon arrival. After beheading and gutting, the bodies are placed in a concrete tank, submerged in fresh water and allowed to soak for 24 h. The fish are removed from the tank and salted. Small fish are split and submerged in brine for 24 h. Solar salt is placed in the abdominal cavities of large fish which are then placed in layers in a concrete tank, each layer receiving a sprinkling of salt. They are then left to pickle for 24 h and receive no effective protection against blowfly attack, being only covered with a mat or piece of sacking. After brining, the small fish are washed in fresh water, placed on interwoven, bamboo mats and taken out to dry. The large fish are removed from the pickle, split and placed back in the (by now blowfly-larvae infested) pickle which is diluted with a few scoopsfuls of fresh water. After 2 h or 3 h the fish are removed from the pickle, washed in fresh water and placed on bamboo mats. Care is taken at this stage to smooth down the flesh surface before placing the fish out to dry. The drying period varies with fish size and environmental conditions. A "poor drying day" might be as short as 3 h, whereas a "good drying day" might last for 8 h. The fish are turned over periodically and during the second day's drying receive a second split. Small fish are generally dried sufficiently within three days, whereas large fish might take from four to six days. The fish are stored indoors overnight at a

temperature of 26°C and relative humidity of 70-90%. At the end of drying, the fish are stacked into piles and stored indoors before being sold in the local market or to a visiting wholesaler.

This standard processing method was used throughout the experiments discussed in this paper, except where indicated otherwise.

Where the processors use household insecticides (Baygon and Startox) to reduce blowfly infestation, the fish are either sprayed or smeared with the insecticide before being placed out to dry, and after the second split the newly exposed flesh is also sprayed or smeared.

3. LOSS ASSESSMENT METHODS

3.1 Assessment of Insect Infestation

In order to obtain a comprehensive picture of insect pest activity and response to loss prevention methods the following observations were taken: (a) development of cultured eggs and larvae; (b) blowfly activity during processing; (c) infestation by blowfly larvae during processing; (d) blowfly egg-laying behaviour.

(a) Blowfly culture

A piece of fish was placed on a bed of salt in a jar and the eggs or larvae were then introduced. The jar was closed with a pad of tissue paper held in place by an elastic band.

(b) Measurement of blowfly activity processing

Blowfly activity in the salting tank and over the drying fish was monitored by taking blowfly counts every 15 min during processing. Supporting environmental data was continuously recorded using a Casella thermohygraph.

(c) Measurement of infestation by blowfly larvae during processing

All fish were carefully examined for the presence of eggs and larvae on arrival at the processors, after soaking in water, after salting and after each day's drying. First instar larvae were simply recorded as being present or absent after removal of the fish from salt, but during drying the level of infestation was graded according to the relative number of larvae present: Grade 1 - no evidence of infestation or only occasional larvae present, Grade 2 - numerous larvae evident on surface or within the flesh.

(d) Measurement of egg-laying behaviour

At the end of each day's drying all fish were examined for the presence of blowfly egg hatches. Each batch was counted and its position marked with a tag.

3.2 Quantitative Assessment of Loss of Edible Solids

A number of factors, all related to the large size of the fish under investigation prevented accurate loss assessment by using the standard FAO method (FAO, 1981).

It was concluded that the most appropriate method of assessing the loss of edible solids from *Arius* during processing was to compare fish which had suffered losses with those that had, by visual inspection, suffered no damage, i.e., those treated with a relatively high concentration of Fastac or fully screened during processing.

Weights were recorded after one day's drying and at the end of drying and, after correction for skin and bone content, the results expressed as percentage loss of edible solids. The method assumes that the fish suffering visual losses dry at the same rate as the fish suffering no visual losses, which was normally found to be the case during the experiments.

3.3 Visual Assessment of Flesh Loss

At the end of drying, a visual subjective assessment was made of the maggot damage to each fish. This was also repeated on some fish after periods of storage.

The visual subjective assessment was made based on the following approximate scale:

Code	Observation	Estimated loss of flesh (%)
O (Zero)	No sign of maggot damage	0
L (Light)	Traces of maggot damage	1
M (Medium)	Obvious signs of maggot damage	1-10
H (Heavy)	Fish badly damaged by maggots	10+

3.4 Assessment of Financial Loss

The processor was asked to estimate the price for which he could sell individual processed fish (ikan jambal) at the local market. This varied from Rp 3 500 (f3) per kilogramme for a fish in excellent condition, to as little as Rp 750 (f0.60) for a badly maggot-damaged fish. On the basis of his grading fish into quality/price bands, an assessment of the financial loss was made.

4. LOSS REDUCTION METHODS

4.1 Extended Salting Times

Large fish (Batch A, > 1 kg weight) were beheaded and gutted, soaked in water for one day and then salted (i.e., a handful of salt placed in the gut cavity and salt sprinkled on each layer of fish in the tank). After one day the fish were removed from the pickle in the salting tank, split and checked for blowfly eggs and maggots. The sample was divided into two on a size basis, so that each sample contained roughly the same size-spread of fish. One half was returned to the liquor in a brining tank, with extra salt placed on the fish, left for one hour, washed in water and placed out to dry. The other half was kept in brine for one-hour duration, and then returned to a brine tank containing the original pickle. They were left for a further 24 h, then checked for eggs and maggots, washed, and placed out to dry.

Small fish (Batch A, < 1 kg gutted weight) were beheaded and gutted, washed and then placed in brine. After one day in brine, half of the fish (sample selected by size) were transferred to drying tables after washing. The remainder were left in the brine for a further day before being checked for eggs and maggots, washed and placed out to dry.

4.2 Screening

One of the two salting tanks at the processors was modified to prevent flies and/or larvae having access to the fish whilst soaking and salting. This was done by building up the cement walls of the tank to give a smooth, level top. A sturdy wooden lid was constructed with insteps so that it fitted the top of the tank. Locally purchased foam rubber was stuck around the edge of this lid in order to give a good seal when placed on the tank. Although the lid was quite heavy, two stones were placed on the lid to improve the seal. To screen the fish whilst drying, three drying screens were constructed from locally available materials (Figure 1).

The fish were processed by the standard method, except some were screened in the salting tank then dried without screening, some were salted without screening but dried for the first two days with screening, and some were screened during both salting and during the first two days drying.

4.3 Pyrethroid Insecticide Treatment

After salting and splitting, the fish were dipped in a solution of insecticide prior to being placed out to dry. When the fish were split, usually during the second day's drying, the newly exposed surfaces were sprayed with insecticide of the same concentration as the dip. Two squirts of the spray were used on each fish and this was found to be equal to about 4 ml. The uptake of insecticide suspension of dipping was about 40 ml/kg of fish.

Two exceptions were made to this procedure during these experiments. In one experiment fish in one salting tank were sprayed with insecticide, and in a later experiment some fish were sprayed with insecticide rather than dipped after salting.

The insecticide formulation used was a water-dispersable preparation of Fastac [(1Rcis)5 and (1Scis)R alpha-cyano-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate]. The concentrations used varied between 0.05% and 0.001%, as discussed below.

5. RESULTS AND DISCUSSION

5.1 Blowfly Eggs and Larvae Culture Results

Two species, *Chrysomya megacephala* (Fab.) and *Lucilia cuprina* (Wied.) were identified as causing heavy infestation during processing and the early stages of storage.

C. megacephala, the "Oriental latrine" fly is commonly found near human dwellings and creates a great nuisance in fish markets and other open places where fish is handled. It is widely distributed over the Oriental and Australasian regions and occurs in neighbouring parts of the Palaearctic region, e.g., China and Japan. *C. megacephala* is not widespread in Africa, but a few specimens, probably introduced by ships, have been identified in Ghana, Senegal, Mali and South Africa. *L. cuprina* is a typically African species, but has spread through southern Asia and has been introduced to Australia and America. Walker and Donegan (1983) have identified *L. cuprina* as being a cause of infestation of drying *Haplochromis* spp. and *Lethrinops* during the season in Malawi.

Under ambient, environmental conditions the eggs of *C. megacephala* had an incubation period of between 6 h and 15 h. The eggs of *L. cuprina* hatched within 12 h. The larval duration was 4-5 days, at the end of which the third instar larvae left the fish in order to pupate. The first emergence of adults from pupae occurred within 9-10 days of the fish being salted in the case of *C. megacephala* and 12-13 days in *L. cuprina*. Wijesundara (1957) estimated that the mean development duration of egg to adult to be 8 days 12 h for *C. megacephala* under controlled, laboratory conditions.

5.2 Extended Salting

The salt concentrations of the pickle in the brining tanks after one-day salting were 26.6% w/v for the small *Arius* tank, and 24.3% w/v for the large *Arius* tank. After two days salting the concentrations had fallen to 19.0% and 19.3% w/v, respectively. The salt content of the flesh of one-day salted fish varied from about 20% (dw) for large fish to about 35% for small fish. The extended salting increased salt levels by about 1-5%.

Table 1 gives the percentage of *Arius* infested with blowfly larvae during salting. In the case of the small *Arius*, protection against infestation was achieved by completely submerging the fish in brine. The large *Arius* were, however, exposed to blowfly attack and extended salting gave no additional protection against infestation.

Drying conditions were poor on the day that the one-day salted large *Arius* were placed out to dry, i.e., low sunlight hours, high humidity. These fish took five days to dry, whereas those salted for two days only took four days to dry under generally good drying conditions. The small *Arius* were all sufficiently dried by the third day of drying, for both one-day and two-day salted fish.

On being placed out to dry, the large fish were subjected to attack by ovipositing blowfly during the first two days drying. Figure 2 shows that extended salting gave no significant extra protection. Figure 2 also shows that only one batch of eggs was laid upon the one-day salted small fish as none of the two-day salted small fish, which probably reflects their higher drying rates.

It can be seen from Figure 3 that blowfly activity over the one-day salted, large fish was relatively high during the first three days of drying. The two-day salted fish showed similar activity for their first two days of drying. The one-day difference can be explained by the poor drying conditions of February 16th, when the fish lost little moisture. Drying conditions improved during the following two days and the similar levels of blowfly activity over both one-day and two-day salted fish suggest extended salting had no significant repellent effect.

Levels of infestation with blowfly larvae were similar in both one-day and two-day salted large fish throughout drying (Figure 4). The visual assessment of losses in the case of larvae *Arius* is shown in Figure 5 both at the end of drying and also after a further three-day storage. After drying, the fish salted for one day (and dried for five days) showed higher damage than those salted for two days (but dried for four days). The higher damage suffered by the one-day salted fish was due to their being exposed to a higher level of blowfly activity while drying on February 16th than the two-day salted fish which remained in the salting tank. However, Figure 5 shows that damage continued during the first three days storage after drying, and at the end of this period the difference between the two groups of fish was relatively small. The small fish suffered no infestation during processing.

The results of this experiment do not agree with the consensus in the literature that salting fish provides protection against blowfly infestation (Kordyl, 1977; Proctor, 1977; FAO, 1981).

Only if this extended salting time had given a very significant increase in fish quality (and hence value) would it have stood any possibility of being implemented. The processor was not willing

to leave the fish for extended periods in the salting tanks as this reduced the quantity of fish that he could process. In addition, a small taste panel (the processor and two Indonesian fish technologists) indicated that some of the small *Arilus* subjected to extended salting were "too salty".

5.3 Screening

Table 2 gives the percentage of *Arilus* infested with blowfly larvae during salting for three batches of fish. The results show that infestation during salting was reduced by screening the tank with a closely fitting lid. After salting, the fish were most susceptible to further infestation during the first two days of drying (Figure 6). Infestation was very much reduced by screening the fish during this period, as seen in Figure 7. The results of the quantitative assessment of loss of edible solids are given in Table 3. The ratio of edible solids (dry weight) to total dry weight used in the calculations, 0.68:1, was the mean value obtained on assessing the ratio for a large number of fish. The effect of screening on the observable losses is shown in Figure 8. This indicates that for effective prevention of losses, the fish need to be screened, both during salting and for at least the first two days of drying.

All of the data obtained relating to losses of fish indicate that screening can be an effective method of loss reduction. The use of drying screens on this occasion was shown to have no significant effect on the drying rates of the fish. Popham (1980) also found that the use of screening, in this case mosquito netting, had no appreciable effect on the drying rate and effectively prevented blowfly from reaching the fish.

There were, however, problems associated with this method of loss reduction. Good hygiene had to be continually maintained, and despite washing the floor, the salting tanks and drying trays prior to use, there was still a small amount of maggot damage to some fish screened throughout processing. This may possibly have been due to flies ovipositing on fish prior to gutting and placing in the salting tank, and this identifies the difficulty in preventing infestation in generally unhygienic surroundings. The second, and major problem, was the size of the drying screens which led to a problem with overnight storage. The processor stacks several drying trays (with the partly dried fish) on top of each other in a store at night. Only two or three drying screens could be stacked together. In addition, their size presented a problem with mobility. In the event of a sudden rain storm, a person could easily carry three drying trays plus fish into shelter, but a single drying screen required two people to move it.

It was observed that the processor readily adopted the salting tank screen and continued to use this in the absence of the research team. However, he would not use the drying screens, except when requested.

5.4 Pyrethroid Insecticide Treatment

The fish were treated with Fastac, a newly developed pyrethroid insecticide. Its high insecticidal activity, coupled with very low mammalian toxicity, makes it ideal for food use and it is gaining wide acceptance for agricultural purposes. It had not previously been used with fish.

In Table 4 the cumulative number of egg batches at completion of drying, the percentage Grade-2 larval infestation after two days drying and quantitative, visual and financial loss assessment data are given for fish treated with several different Fastac concentrations and for untreated controls. Figure 9 shows the change in cumulative number of egg batches during drying for three Fastac concentrations, Figure 10 shows the change in percentage Grade-2 infestation and Figure 11 the blowfly activity during drying for the experiment with a Fastac concentration of 0.0125%.

Fastac was effective in reducing, but not preventing, egg-laying when used as a dip in concentrations from 0.05% to 0.00625%. No Grade-2 larval infestation was present after two days drying for dip concentrations from 0.05% to 0.003%, however, Grade-2 infestation was evident on the morning of day 2 for the 0.002% and 0.001% concentrations. The repellent effect of Fastac was particularly noticeable when blowfly activity was high, as with Batch C (Figure 11). Fastac was clearly demonstrated to be larvicidal. Fish which were heavily infested during salting showed no further larval activity after being dipped in Fastac concentrations from 0.05% to 0.003% and dead first instar larvae were observed on the fish. Loss assessment of edible flesh by the method described earlier gave an average loss of 9% ($\pm 3\%$, 95% confidence limit) for untreated fish. Visual assessment demonstrated that dipping in Fastac prevented losses during drying and concentrations as low as 0.001%. Although residue levels are not yet available, the concentrations for Fastac used are lower than has previously been reported for any other effective insecticide treatment of drying fish.

Application of Fastac as a spray prevented infestation during salting, but did not give continued protection during drying presumably because of uneven application and lower uptake by the fish.

6. CONCLUSIONS

- (1) Salting, using a pickla cura method, does not protect *Arius* spp. from infestation by *C. megacephala* and *L. cuprina* during processing.
- (2) Guarding the salting tank with a closely fitting cover prevented blowfly infestation during salting and was readily adopted by the processor.
- (3) Screening during drying, though effective in reducing infestation and losses, proved inconvenient and unacceptable to the processor.
- (4) Dipping the fish in Fastac protected fish against blowfly infestation and reduced losses even when used at very low concentrations.
- (5) A combination of screening during salting and Fastac dipping before drying proved the most effective procedure for preventing blowfly infestation and reducing losses during processing.

7. REFERENCES

- FAO, The prevention of losses in cured fish. FAO Fish.Tech.Pap., (219):87 p. Issued also in 1981 French.
- Kordyl, E., Some protective measures against insect infestation of dried fish in Africa. In 1977 Proceedings of the Conference on handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 313-4
- Popham, E.J., Inexpensive means of controlling insect infestation of dried fish from Lake Chilwe, 1980 Malawi. Luso.J.Sci.Technol., 1980
- Proctor, D.L., The control of insect infestation of fish during processing and storage in the 1977 tropics. In Proceedings of the Conference on handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 307-11
- Walker, D.J. and L. Donagan, Personal communication obtained in Malawi 1983
- Wijesundara, D.P., The life history and bionomics of *Chrysomya megacephala* (Fab.). Ceylon J.Sci. 1957 (B Pt.3), 25:170-85

Table 1

Percentage of *Arctus* infested with blowfly larvae during salting

Batch	1-day salting	2-day salting
A - large	25	46
A - small	0	0

Table 2

Percentage of fish infested with larvae during selting

Batch	Screened	Unscreened
B	15	100
C	0	25
D	0	100

Table 3

Percentage loss of edible solids for screened and unscreened *Arctus* relative to loss for fish screened in salting tank and for first two days drying taken as zero

	Percentage loss of edible solids	
	Batch B	Batch C
Unscreened fish - controls (6 fish)	9	13
Fish screened in salting tank only (6 fish)	7	12
Fish screened for first two days drying only (3 fish)	19	16
Fish screened in salting tank and for first two days drying (6 fish)	0	0

Table 4
The effect of insecticide on A_{ring} losses - experimental detail and results

Batch	Treatment	No. of fish	Cumulative No. of egg batches at end of drying	Grade 2 larval infestation after 2 days drying (%)	Quantitative assessment of % loss of edible solids ^{a/}	Visual assessment of loss				Financial loss ^{a/} (%)
						Zero	Low	Medium	High	
p ^{b/}	0.05% Fastac dip	8	0	0	0 ^{a/}	8	0	0	0	-
	None	6	2	66	5	0	2	2	2	-
E	0.5% Fastac	10	0	0/0 ^{a/}	-	10	0	0	0	-
	spray tank and dip	8	0	0/0 ^{a/}	-	3	0	0	0	-
	dip only	9	1	10/40 ^{a/}	-	5	4	0	0	-
	spray tank only	9	8	0/66 ^{a/}	-	5	1	3	0	-
F	0.025% Fastac dip	10	6	0	-	10	0	0	0	0 ^{a/}
	None	10	46	40	-	0	0	8	2	26
G	0.0125% Fastac dip	10	1	0	0 ^{a/}	10	0	0	0	0 ^{a/}
	Baygon spray	10	32	17	7	2	2	6	0	6
	None	10	32	17	11	2	1	7	0	20
H	0.00625% Fastac, one dip	10	0	0	0 ^{a/}	10	0	0	0	0 ^{a/}
	0.00625% Fastac, two dips	10	1	0		10	0	0	0	
	None	10	23	60	10	2	1	5	2	39
I	0.003% Fastac dip	6	6	0/0 ^{a/}	0 ^{a/}	6	0	0	0	-
	0.002% Fastac dip	6	5	17/0 ^{a/}		6	0	0	0	-
	0.001% Fastac dip	6	2	33/0 ^{a/}		6	0	0	0	-
	0.002% Fastac spray	6	11	33	13	2	0	4	0	-
	None	6	4	33	5	2	2	2	0	-

a/ The loss for the Fastac-treated samples is taken as zero

a/ The loss for the Fastac-treated samples is taken as zero
b/ Some of the same batch of fish were used in the scanning experiments described above
c/ First figure is after 2 days drying, second figure after 3 days drying
d/ First figure after 1½ days drying, second figure after 2 days drying

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d/ First figure after 1½ days drying, second figure after 2 days

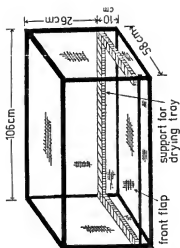


Figure 1 Drying screen

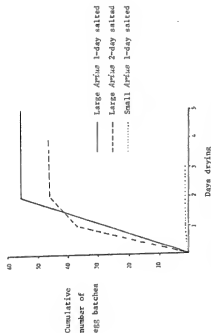


Figure 2 The effect of extended salting upon egg-laying for Batch A fish

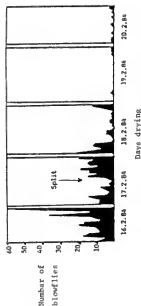


Figure 3a Blowfly activity on 1-day salted, large Arius during drying (Batch A)

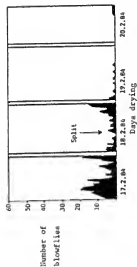


Figure 3b Blowfly activity on 2-day salted, large Arius during drying (Batch A)

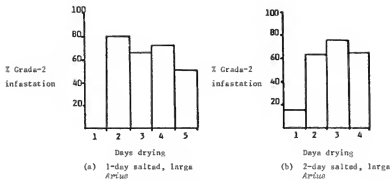


Figure 4 Effect of extended salting upon the larval infestation of Batch-A *Arctus*

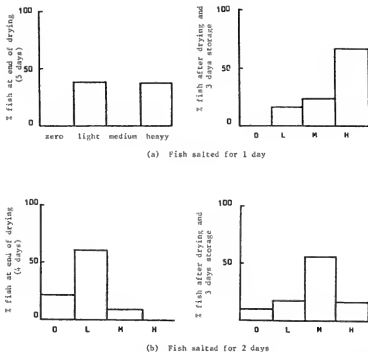


Figure 5 Visual assessment of losses - affect of extended salting time on large *Arctus* (Batch A)

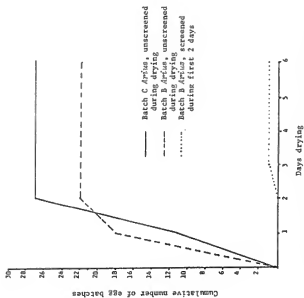


Figure 6 The effect of screening upon egg laying during drying

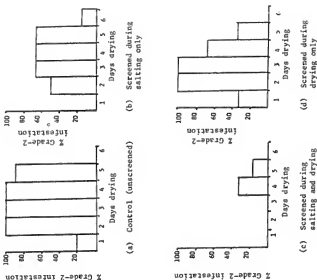


Figure 7 Effect of screening upon larval infestation of *Arizus*

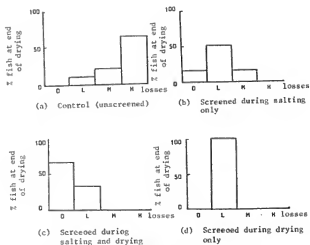


Figure 8 Visual assessment of losses - effect of screening *Arius*

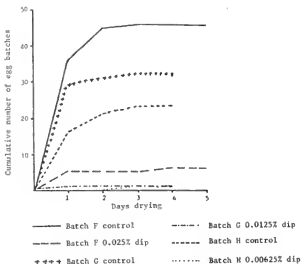


Figure 9 The influence of Fastac concentration on egg-laying

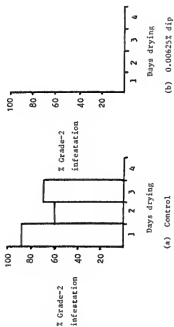


Figure 10 The effect of 0.00625% Fastac dip upon larval infestation of *Arizus* during drying (Batch H)

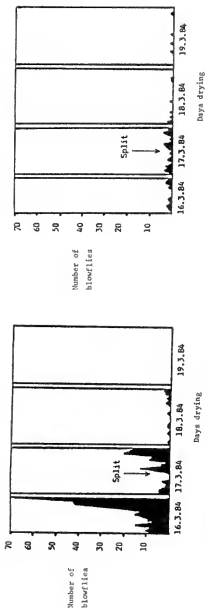


Figure 11a Blowfly activity on untreated *Arizus* (Batch G)

Figure 11b Blowfly activity on *Arizus* treated with a 0.00625% Fastac dip (Batch G)

COMPARISON OF SUN-DRIED AND OVEN-DRIED
SALTED FISH

by

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ABSTRACT

Traditional fish salting and drying processes in Malaysia give products of variable quality. Sun drying and oven drying of *Johnius soldado* were compared organoleptically after the fish had been eviscerated, washed and salted at 10, 20, 30, 40% w/w. Sun drying was at ambient (26.7°C and 95.9% RH) oven drying at 45°C with air speed 2.5 m/sec. Drying times were up to 40 h for sun drying and only 8 h for oven drying. The 20% salted oven dried sample was judged the best.

1. INTRODUCTION

The technique of salting and drying fish has been used for a long time in Malaysia. Fish provides approximately 49% of the total animal protein consumed and 12% of the total protein intake. About 5-10% of the total catch landed in the 1970s were salted and dried. On a finished product basis this amounts to 77% of the total edible output of processed fish (Abdullah and Idrus, 1978). Dry-salted fish forms a relatively cheap source of high quality protein especially for those residing in the rural areas.

The techniques involved in salting and drying in Malaysia are rather simple. Except for the concrete vats for salting and the bamboo or wooden platforms for sun drying, no other equipment is used. The fish are usually washed before salting and for big fish like red snapper (*Lateolabrax* spp.) and catfish (*Pachyurus kasugata*), the gills and gut contents are removed, whilst smaller fish such as herring and scad are not eviscerated. After evisceration, the fish may or may not be split along the dorsal line. The eviscerated fish is usually not cleaned before salting. The amount of salt used and the salting and drying times depend on the size of the fish.

Salting is carried out in concrete vats by arranging the fish in alternate layers with coarse rock salt. In order to prevent the fish from floating, weights are placed on the surface. The vats are housed within sheds to avoid excessive sun and rain and the length of salting times ranges from 1-5 days. When salting is complete the fish are removed and washed in the sea or brackish water to remove adhering salt. Sometimes the fish are soaked for 10-30 min to remove excess salt.

Sun drying is the common practice and no mechanical driers or any other forms of accelerated drying methods are used. The fish are spread out on bamboo platforms or on the ground and are arranged so as to facilitate drying. Bigger fish are split open and flattened out and may be hung by the tail to dry for a day before being transferred to the drying platforms. In some areas along the east coast, pepper is rubbed into the gut cavity and cut surface of the fish to prevent spoilage. This procedure is only carried out for fish that will fetch a good price. On the west coast, powdered alum is used instead. Insect infestation becomes more serious in damp weather.

During drying the fish are turned over once or twice daily. Processors detect the stage of drying usually by pressing the fish with the hand, by judging the colour and appearance of the skin and by the condition of the eye. If the eye is clear and not watery, then the fish is considered sufficiently dried. In the last stage of processing, the dried fish are packed into cardboard boxes which may be lined with polythene sheets before dispatch to the wholesaler. Storage conditions are poor and no attempts are made to maintain the quality of the fish during transit.

The lack of technology associated with production of dry-salted fish in Malaysia means that the products are very often of poor quality. In addition the poor quality of the salt used often imparts a rough, whitish crust to the surface of the fish, undesirable brown discolouration and gives rise to unpleasant bitter flavours and tough texture (FAO Fish. Circ. 336, 1976). Bacteria which contribute to spoilage in dry-salted fish cannot survive below a water activity (a_w) of 0.75 (Beatty and Fougeres, 1957). Malaysian dry-salted fish have 0.75-0.86 a_w . In a hot, damp tropical climate (average temperature 26-28°C, with a relative humidity reaching 96-97%) dry-salted fish are highly susceptible to spoilage by either micro-organisms or infestation by flies. During the drying stage, no precautions are taken to prevent insect infestation so that flies lay eggs and subsequently the larvae tunnel into the flesh causing putrefaction and extensive damage.

Failure to recognize the poor fish make poor products of any kind is a common error among fish processors in Malaysia. Usually, fish considered to be unsuitable for distribution as fresh fish are diverted to the salting and drying process.

2. MATERIALS AND METHODS

The species *Johnius beladano* was used as it is the most commonly available dry-salted fish. The fish were descaled, eviscerated and washed in clean water. They were then placed in alternate layers with salt in a salting vat, covered with a plastic sheet and stored at room temperature (24°C). Salt ratios of 10, 20, 30 and 40% (w/w) were used.

For sun drying, the fish were dried to constant weight by suspending by the tail from hooks inside a drying chamber, consisting of racks supported by a metal frame covered with wire mesh. The fish were kept at a distance 15 cm apart in a staggered formation to ensure efficient circulation of air. The average temperature of the atmosphere during the drying period was 26.7°C and the relative humidity was 95.9%. Average wind speed was 1 m/sec. The oven-dried fish were dried in a forced-air cabinet drier (Apex, U.K.) at 45°C using an air speed of 2.5 m/sec. The fish were placed on their sides on wire mesh and were turned over frequently for more uniform drying. The dried fish were kept in sealed polyethylene bags until ready for sensory evaluation.

For sensory evaluation the fish flesh was cut into cubes (15 cm x 1.5 cm x 1 cm) and fried in oil at 200°C for 2-3 min. before tasting. The panelists assessed for the appearance, texture, flavour, presence of undesirable odour and overall acceptability on a hedonic rating scale of 9 maximum of five points for a favourable response. The results were analysed using the Least Significant Difference method.

3. RESULTS

For salting, samples with lower salt contents (10 and 20%) took 24 h to reach a constant salt level. Those in the 30 and 40% salt ranges took over 36 h. A maximum salt level was achieved in the 30% salt sample. Moisture losses were correspondingly highest for the 30 and 40% salt samples (Yu, Sim and Idrus, 1982).

For sun drying one of the problems encountered was the fluctuation in weather conditions. Samples took up to 40 h to reach a constant moisture level. However, using a forced-air dehydrator, this was scaled down to 8 h and a lower moisture content of 18-21%. (Yu, Sim and Idrus, 1982).

From sensory evaluations, the 20% salted and oven-dried sample was judged the best. This is followed by the 10% salted oven-dried sample. The 30 and 40% salted samples were significantly less acceptable (Table 1).

However, for sun-dried samples, the 30% salted fish was the most acceptable, followed by the 40% salted sample. The 10 and 20% samples were at least acceptable.

All the samples contained similar salt levels irrespective of the drying method (Table 2). The sun-dried samples had higher and more inconsistent moisture contents, especially for the 10 and 20% salted samples.

For the oven-dried samples, the 30 and 40% salted sample were less acceptable mainly due to the salt causing an unattractive appearance and undesirable texture and flavour (Table 1). But this high salt content can be better tolerated provided that moisture levels are kept low. This is exemplified by the 10 and 20% salted sun-dried samples, where, although salt levels are low, moisture levels are high. This causes the development of bad odour and flavour. At the 30 and 40% salt level for both processes, samples that were sun dried were more acceptable, mainly due to a better appearance and a lesser dehydrating effect since the sun-dried sample contained more moisture.

The process of producing salted fish by sun drying is difficult to control in view of the Malaysian climate, with frequent rains and a varied and humid climate. The present dependence on sun drying results in excessive spoilage. It is suggested that controlled environment be used in order to process for a uniform dry-salted product.

Table 1

Statistical analysis of the scores by Friedman's two way analysis of variance by rank

(%) Salt content	Appearance	Texture	Flavour	Undesirable odour	Acceptability	Total score
Oven-dried						
10	42	38	36	50	44	210
20	44	46	47	48	47	232
30	36	36	34	48	36	190
40	31	33	32	45	32	173
Sun-dried						
10	34	28	29	31	29	151
20	36	24	33	34	39	166
30	44	33	41	45	43	206
40	40	33	34	46	37	190
χ^2_r	14.113 ^{a/}	14.182 ^{a/}	14.244 ^{a/}	19.192 ^{b/}	17.647 ^{a/}	19.637 ^{b/}

^{a/} p = 5%

^{b/} p = 1%

Table 2

Moisture and salt contents of dry-salted fish

Salt level	(%) Moisture content	(%) Salt content
Oven-dried		
10	20.95	10.52
20	20.00	12.02
30	18.00	20.59
40	19.50	20.69
Sun-dried		
10	38.00	9.70
20	29.00	12.60
30	24.00	19.20
40	24.00	19.80

5. REFERENCES

- Abdullah, M.I. and A.Z. Idrus. The fish processing industry in Peninsular Malaysia. Proc. IPFC, 1978 18(3):45-60
- Bastty S.A. and H. Pougere. The processing of dried-salted fish. Bull. Fish. Res. Board Can., (112):54 p. 1957 Issued also in French
- FAO. Code of practice for salted fish. FAO Fish. Circ., (336):54 p. Issued also in French and Spanish 1976
- Yu, S.Y., C.L. Siew and A.Z. Idrus. The application of technology to the processing of dry-salted fish in Peninsular Malaysia: comparison of sun-dried and oven-dried fish. J. Food Technol., 17(2):211-8 1982

NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF
DRIED FISH PRODUCTS IN THAILAND

by

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ABSTRACT

Since roller dried fish (RDF) and fish protein concentrate type B (FPC-type B) are new products, their quality and safety assurance were determined by rat bioassay. Thirty-two weanling male Sprague Dawley rats were used for nutritional evaluation, using casein as the reference protein. All diets were adjusted to $10 \pm 0.3\%$ protein level and the assay period lasted for four weeks. RDF from threadfin bream showed the highest protein quality in terms of protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and digestibility (D) with values of $2.71 \pm 0.13\%$, 81.24%, 86.25% and 94.19%, respectively. However, RDF from sardine showed a nutritional value similar to casein. Three-month toxicological studies were performed in another experiment using 120 rats. No significant differences were noted in either body weight gains or food consumption. The average food efficiency was higher in animals fed test diets than those on the stock ration. No abnormalities were noted among animals in hematological studies except for some variations in the serum alkaline phosphatase activity in the blood chemistry studies. Histopathological findings revealed no specific lesions except for some congestion and calcification at the corticomedullary junction of the kidneys in animals fed 16% and 20% of RDF from threadfin bream, 24% of RDF from sardine and a few of the controls. In addition, gastrointestinal lesion appeared in animals fed 16% and 24% of RDF from threadfin bream as edema and congestion at the submucosal surface of the Payer's Patch. This short-term toxicity study revealed no significant evidence that would impose any hazard with respect to longevity, carcinogenicity, dominant lethal, reproductive or biophysiological function.

1. INTRODUCTION

In Thailand, over 50% of the animal protein consumed is derived from fish. Recent catches have yielded increasing quantities of small fish. To obtain maximal nutritional benefit from fish in Thailand requires conversion of this type of catch into fish products. Two of the most successful fish products made from small fish are roller dried fish and fish protein concentrate. These two products have been produced and consumed in Europe for several years and have been successfully introduced by the Food and Agriculture Organization of the United Nations into several other countries. Thailand, with the support of the Food and Agriculture Organization, undertook the task of incorporating these products into traditional Thai dishes and conducting acceptability test for the two new products, roller dried fish and fish protein concentrate.

The purpose of this study is to investigate the nutritional quality and the safety of the products before introducing to the consumers.

2. MATERIALS AND METHODS

2.1 Quality Assurance

RDF from threadfin bream and sardine and FPC-type B were taken for proximate analysis according to standard AOAC procedures (AOAC, 1975). Then, all samples were formulated to have the same chemical composition and the protein levels were adjusted to $10 \pm 0.3\%$ including casein, the reference protein. A slight modification of diet composition was performed by using soy oil instead of cottonseed oil and corn starch/mucrose (1:1) was used as the carbohydrate sources. Amino acid and vitamin content of dried fish products were analysed by Department of Nutrition and Department of Medical Science, Ministry of Public Health, respectively. The mineral contents were analysed by Department of Medical Science, Ministry of Public Health and Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University.

Animals used in this study were 32 weanling male Sprague Dawley rats, divided into four groups, one control and three test groups. All animals were housed individually in stainless steel metabolic cages in the controlled experimental room. The experimental period for PER and nitrogen balance studies lasted for four weeks and were performed at the same time and the PER corrected and calculated according to Campbell (1960). Daily food intake and weekly body weights were recorded. Weekly urinary and faecal nitrogen belonging to the appropriate test-diet groups were collected and analysed by macro-kjedahl method. The excretion of urinary and faecal nitrogen of rat fed a protein-free diet was carried out by separate experiments. The following equations to yield the endogenous urinary and faecal nitrogen derived from protein-free diet; experiments were performed by Paramadillo *et al.* (1979).

$$U_k = 0.167 X + 7.970$$

$$F_k = 0.047 X - 0.013$$

where U_k = endogenous urinary N (mg/rat/day)

$$F_k = \text{endogenous faecal N (mg/rat/day)}$$

$$X = \text{mean of body weight (g)}$$

2.2 Toxicity Testings

Animals: 60 male and 60 female Sprague Dawley rats at weanling age were divided into ten groups, one control and nine test groups and fed *ad libitum* for 12 weeks as scheduled in Table 5. Daily food intake and weekly body weights were recorded. The food efficiency ratio was calculated from this data. At the end of the study the animals were anesthetized by ether inhalation and blood collected by heart puncture. The hematological values, blood urea nitrogen and serum enzymes, such as alkaline phosphatase, serum glutamic pyruvic transaminase and serum oxaloacetic pyruvic transaminase were estimated. The animals were sacrificed. Organ weights, e.g., heart, liver, kidney, spleen, lungs, brain, adrenal gland, pituitary gland, thyroid gland and testis or ovary of the animals at each point of sacrifice were determined and expressed as the percentage of their body weights. The above tissues including stomach, small intestine, pancreas were fixed in 10% buffered neutral formalin and sections stained with haematoxylin eosine and examined under light microscope.

3. RESULTS AND DISCUSSION

3.1 Quality Assurance

The proximate and amino acid analyses of all the dried fish products are given in Tables 1 and 2. The protein content and amino acid content of RDF were higher than those of FPC-type B. The amino acid analyses of all the products indicated that valine was the limiting amino acid. However, RDF from both types of fish contained large amount of lysine, methionine, leucine, arginine, alanine, aspartic acid and glycine. Lysine and methionine of RDF were higher than those of casein, while those from FPC-type B were relatively the same. FPC-type B had much higher fat content than RDF. For the vitamins, both RDF and FPC-type B were rich in vitamins A, B₁, B₂ and E (Table 3). FPC-type B had higher content of vitamin A and E than RDF made from the same fish and was an excellent source of many essential minerals, particularly calcium, phosphorus and iron, including fluorine (Table 4).

The results on protein evaluation using rat bioassay in N-balance experiments are given in Table 6. RDF from threadfin bream was the best product giving highest PER value of $2.71 \pm 0.13\%$ and was significantly better than RDF from sardine, casein and FPC-type B. However, N-balance studies of RDF from sardine showed similar good results to those from threadfin bream.

3.2 Toxicity Testings

The composition of the diets used in this experiment is presented in Table 7. The average body weight gain, average food consumption and food efficiency are presented in Table 8. The food efficiency was higher in animals fed the tested diets than those fed the stock ration. The results of the hematological and blood chemical value of the tested animals were within the normal range for the animals of this age and strain.

Gross pathological examination of the controls and tested animals fed different diets showed no significant difference in the organ weight, organ to body weight ratio and organ to brain weight ratio. No histopathological lesions were observed on the heart, liver, kidney, spleen, lung, stomach, small intestine, pancreas, brain, testis or ovary, adrenal gland, pituitary gland and thyroid gland. However, there were some congestion and calcification at the cortico-medullary junction of the kidney in animals fed 16% and 20% roller dried fish from threadfin bream, 24% roller dried fish from sardine and few of the controls. In addition, gastrointestinal lesion appeared in animals fed 16% and 24% roller dried fish from threadfin bream as edema and congestion at the sub-mucosal surface of the Payer's Patch.

Table 1

Proximate analyses of dried fish products (wet weight basis)

Food product	Proximate analysis (%)					
	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate ^{a/}
Standard casein	12.42	82.64	0.87	1.67	0.41	2.40
RDF (sardine)	5.55	75.20	5.26	5.19	0.12	8.80
RDF (threadfin bream)	6.59	74.51	7.41	5.15	0.10	6.34
FPC-type B	2.32	64.15	11.15	22.27	2.06	0.11

^{a/} Expressed as: 100-(protein + fat + ash + moisture)

Table 2

Amino acid content of standard casein and dried-fish products^{a/}

Amino acid (g/100 g product)	Casein	RDF		FPC-type B
		Sardine	Threadfin bream	
Tryptophan	1.311	0.961	0.955	0.677
Threonine	3.848	4.045	3.776	3.510
Isoleucine	3.700	3.674	3.470	2.717
Leucine	5.127	6.419	5.826	4.737
Lysine	6.466	8.225	8.590	6.299
Methionine	1.850	2.195	2.045	1.748
Cystine	0.710	0.687	0.681	0.456
Phenylalanine	3.368	3.531	4.348	2.436
Tyrosine	3.647	2.384	2.217	1.916
Valine	3.655	3.961	3.469	3.066
Arginine	2.982	5.280	5.180	5.126
Histidine	2.410	2.802	1.832	1.932
Alanine	2.161	4.382	4.075	3.790
Aspartic acid	5.067	7.417	6.898	5.707
Glutamic acid	17.387	11.818	11.036	9.259
Glycine	1.296	3.549	3.035	4.141
Proline	8.776	2.624	2.645	2.881
Serine	4.170	2.935	2.680	2.544

^{a/} Analysed by Department of Nutrition, Ministry of Public Health

Table 3

Vitamin content of dried fish products^{a/}

Vitamin (mg/100 g product)	RDF		FPC-type B
	Sardine	Threadfin bream	
A	0.10	0.06	0.42
B ₁	0.02	0.18	0.10
B ₂	0.50	0.15	0.27
E	1.25	3.16	3.36

^{a/} Analysed by Department of Medical Science, Ministry of Public Health

Table 4

Mineral content of dried fish products

Mineral ^{a/} (mg/100 g product)	RDF		FPC-type B
	Sardine	Threadfin bream	
Zinc	4.52	3.50	6.95
Iron	5.66	1.59	10.83
Copper	0.80	0.40	0.30
Phosphorus	7.08×10^2	6.92×10^2	2.07×10^3
Calcium	9.70×10^2	5.14×10^2	2.40×10^3
Lead	0.20	0.21	0.00
Tin	20.35	23.97	23.90
Cadmium	0.05	0.02	0.05
Mercury	0.01	0.02	0.01
Fluorine ^{b/}	12.00	8.00	108.00

^{a/} Analysed by Department of Medical Science, Ministry of Public Health

^{b/} ppm, Analysed by Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University

Table 5

Type and level of foods used in the toxicity test

Group	Kind of diet	Dietary level	Number of animals	
			Male	Female
0	Stock ration ^{a/}	-	6	6
A ₁	RDF (threadfin bream)	16 %	6	6
A ₂		20 %	6	6
A ₃		24 %	6	6
B ₁	FPC-type B	18 %	6	6
B ₂		23 %	6	6
B ₃		28 %	6	6
C ₁	RDF (sardine)	16 %	6	6
C ₂		20 %	6	6
C ₃		24 %	6	6

^{a/} Produced by Biological Test Section, Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand

Table 6

Nutritional evaluation of tested foods

Group/diet	Weight gain (g)	PER ^{a/}	Corr. PER ^{b/}	NPU ^{c/}	BV ^{d/}	D ^{e/}
Casein	135.95 ± 12.90 ^{f/}	3.43 ± 0.18	2.50 ^{g/}	76.41	81.40	93.88
RDF (sardine)	140.62 ± 18.84	3.45 ± 0.18	2.51 ± 0.13 ^{g/}	80.09	85.46	93.72
RDF (threadfin bream)	167.32 ± 14.02	3.73 ± 0.18	2.71 ± 0.13 ^{g/}	81.24	86.25	94.19
FPC-type B	136.80 ± 11.76	3.38 ± 0.16	2.47 ± 0.12 ^{g/}	76.00	83.81	90.69

^{a/} Protein Efficiency Ratio (PER) = weight gain (g)/protein consumed (g)

^{b/} Corrected PER as adjusted to 2.50 for casein

^{c/} Net Protein Utilization (NPU) = retained nitrogen/intake nitrogen) 100

^{d/} Biological Value (BV) = (retained nitrogen/absorbed nitrogen) 100

^{e/} Digestibility (D) = (absorbed nitrogen/intake nitrogen) 100

^{f/} Mean ± standard deviation

^{g/} Indicated statistical difference, P < 0.05

Table 8

Data on body weight gain, average food consumption and average food efficiency of rats in 12 weeks

Group	Sex	Body weight gain (g) in 12 weeks	Average food consumption (g/rat/wk) in 12 weeks	Average food efficiency in 12 weeks
0	M	348.87	156.15	19.74
	F	171.21	123.27	12.68
A ₁	M	329.79	142.26	19.88
	F	205.77	118.53	15.26
A ₂	M	347.31	142.68	21.23
	F	198.96	113.01	15.13
A ₃	M	354.61	139.92	22.35
	F	213.07	113.55	16.81
B ₁	M	359.45	151.92	21.15
	F	193.25	115.52	14.53
B ₂	M	345.94	151.22	20.34
	F	206.97	124.73	14.68
B ₃	M	364.34	149.71	21.78
	F	201.57	119.17	15.19
C ₁	M	346.79	145.12	20.82
	F	195.00	115.21	14.70
C ₂	M	349.50	144.50	20.92
	F	183.68	112.62	14.04
C ₃	M	347.35	143.34	21.93
	F	186.25	110.59	14.25

M : Male rats

F : Females rats

Food efficiency = gram weight gain/100 gram food consumed

4. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, 1975 D.C., AOAC, 12th ed.
- Campbell, J.A., Evaluation of protein in foods for regulating purpose. J.Agric.Food Chem., 8:327 1960
- Parmadilok, P., et al., Determination of urinary and faecal nitrogen of albino rat fed a protein-free diet in terms of linear equation. Food, 11(4):277-82

STUDIES ON THE PREPARATION OF SALTED AND DRIED MINCES FROM
THREADFIN BREAM (*Nemipterus japonicus*) AND
INDIAN OIL SARDINE (*Sardinella longirostris*)

by

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ABSTRACT

Selting of minced meat, a better alternative to traditional salting of whole fish, was tried with threadfin bream and Indian oil sardine. Different salt-mince ratios and drying conditions were used to get an acceptable product. Shelf life of the products, their acceptability and economics are discussed in a local recipe.

1. INTRODUCTION

The idea of deboning fish and converting the meat into comminuted mince is innovative, as minced fish permits the use of less expensive, underutilized species and provides greater yield of edible flesh. It also offers unlimited opportunities for processors to generate a wide range of new fish products suited to consumers with varied diets.

The preservation and storage of minced fish is as much a problem as that of fresh fish. During the last fifteen years, many researchers have studied salting as a technique for the preservation of minced fish under tropical conditions, with emphasis on salt-mince ratio. The suitability of different fish species for producing salted mince, their proximate composition and production on a pilot plant scale have been reported (Del Valle and Nickerson, 1968; Del Valle and Gonzalez-Inigo, 1968; Del Valle et al., 1973, 1976). Mendelsohn (1974) and Baraquot (1974) have reviewed the various rapid salting techniques for fish and fish mince. Observations on and modifications of salting processes for minced meat of lean fishes have recently been reported (Wojtowicz, Fierheller and Regier, 1975, 1978; Wojtowicz et al., 1977; Bligh, 1976; Young et al., 1979; Bligh and Duclos, 1982). Most of the researchers have repeatedly stressed the importance of selting fish mince, highlighting its advantages over conventional salting methods.

In the present study, the suitability of a lean fish, threadfin bream (*Nemipterus longirostris*), and a fatty fish, oil sardine (*Sardinella longirostris*), as raw materials for producing salted mince was investigated and their shelf life at ambient temperature (30°-35°C) assessed. Preliminary taste panel tests on fish mince prepared from salted mince of both species have been conducted and economics of the process discussed.

2. MATERIALS AND METHODS

2.1 Fishes Used and Separation of Meat

The fishes used in the study were Indian oil sardine (*Sardinella longirostris*) and threadfin bream (*Nemipterus japonicus*). Samples which were uniced at the time of landing were brought to the laboratory, washed and iced immediately. The meat was separated by using a pounding type meat picking machine, which had 6.5 mm perforations in its separating disc. The separated meat was minced in a power-driven meat mincer using a disc with 5-mm holes.

2.2 Preparation of Salted and Dried Mince

The following standardized method was followed for the preparation of salted and dried minces. The fish meat separated was first mixed with locally available refined table salt in salt to mince ratios of 1:2, 1:3 and 1:4. This mixture was held at ambient temperature (30°-35°C) for 1.5 h in the case of threadfin bream and 2 h in the case of oil sardine, after which the separated brine was drained off and the mince, wrapped in muslin cloth, taken through a hand-operated screw press for expelling the remaining brine. Circular and rectangular brickets, each weighing about 45-50 g were made out of this mince, using suitable aluminium moulds. Half the number of brickets were sundried, while the other half were dried in a Kilburn oven with facility for air circulation. They were then packed in polythene bags, heat sealed and stored at ambient temperature (30°-35°C) and humidity (68-72%). In the case of oil sardine mince, water bleaching before mixing with salt and warming of

salt-mince mixture before pressing were carried out. Shaping into bricks was found to be unsatisfactory for salted mince of oil sardine, hence the final product was prepared in the form of shreds only. For purpose of comparison, shreds were, therefore, prepared from threadfin bream also. Since sundrying of sardine mince was found to take too long, it was subsequently subjected only to oven drying.

The percentage yields of dressed fish, picked meat and the final product in relation to whole fish, dressed fish or picked meat, as well as time taken for drying of salted minces at 50°C in an oven and under the sun were recorded.

2.3 Analyses

Samples drawn at monthly intervals were analysed for biochemical, microbiological and organoleptical parameters. However, the analyses for determining pepsin digestibility and available lysine were conducted only twice, once immediately after the preparation of the products and the next time after their storage over three months.

The chemicals used for analysis were of either 'analytical' or 'GR' grade, obtained from either BDH or Sarabhai, India. Fluorodinitrobenzene was obtained from Sigma chemicals, USA and DNP-lysine from BDH Ltd., Poole, England. The media and pepsin used in the study were obtained from Hindustan dehydrated media, Bombay.

The proximate composition of the raw material and the final product was determined according to the methods described in AOAC (1975). The estimation of sodium chloride was done according to the method given by FAO (1981). Trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) were estimated as per the method described by Beatty and Gibbons (1937). The 2-thiobarbituric acid (TBA) value was determined by the method of Yu and Sinnhuber (1957). Pepsin digestibility was estimated as described in AOAC (1975). Available lysine was determined by the method of Carpenter (1960). Moulds and halophilic bacteria were determined according to the methods recommended by APHA (1976). The rehydration ratio of the samples was determined by the method described by von Loescke (1955).

2.4 Organoleptic Assessment

For this purpose, the minces were first desalted by soaking in water in 1:4 ratio for 30 min. The desalted minces were used for Kheema preparation, following the procedure described by Revenkar, Naider and Baliga (1979). Kheema prepared from frozen minced meat was used as control. The preparations were tested for overall acceptability by twelve experienced panelists. Economics of the process has been worked out.

3. RESULTS AND DISCUSSIONS

The initial good quality of the raw material is one of the foremost requirements for the preparation of any product. The two fish species used in the present study were found to be slightly low in their initial quality, as indicated by their TMA-N and TVB-N values (2.38 mg% TMA-N and 13.7 mg% TVB-N for threadfin bream and 1.94 mg% TMA-N and 7.84 mg% TVB-N for oil sardine). This is understandable, since the fish obtained from the local landing centre had been brought uniced from the fishing ground. The percentage yields of dressed fish, picked meat and salted mince in relation to whole fish, dressed fish or picked meat in respect of the two species of fishes studied are shown in Table 1, while the proximate composition details of picked meat and final product are given in Table 2. There was no significant difference in the percentage yield of picked meat between oil sardine (37.3%) and threadfin bream (36%). However, in their proximate composition, oil sardine showed significantly higher percentage of protein than threadfin bream. By virtue of its high fat content, the oil sardine meat was found to be not quite satisfactory in its appearance and required water bleaching in order to improve its colour and reduce the fat content. By water bleaching, the fat content was reduced from 11.7% to 4.4%. The proximate composition of water-bleached meat is also shown in Table 2.

3.1 Salting of Fish Mince

The condition under which salting is done is highly important from the stand point of stabilization of protein components, particularly in minced fish meat. Complete salting will precipitate protein into a fibrous, opaque and shrunken form, releasing a major part of tissue water. This is possible only if enough salt is available and also by 'fast' salting. Fast salting results in direct precipitation of protein without intermediate gel formation (Mendelsohn, 1974). In this study, among the three salt to mince ratios tried, the 1:3 ratio was found to be the best, since the separation of brine was maximum. The percentages of brine separated were 54 and 52 in the case of threadfin bream mince and oil sardine mince, respectively (Table 3). When one part of salt was mixed with three parts of threadfin bream mince of 81.2% moisture, the mixture was found to contain 25% salt, 61% water and 12.4% fish solids. The amount of salt related to the water phase of the mixture (29%) was well over saturation. In fact, by raising the salt content further, by resorting

to 1:2 salt to mince ratio, oversaturation occurred and the product had undesirable qualities. These results agree very well with the results obtained by Neudersohn (1974) and Wojtowicz *et al.* (1977). Similar results were obtained for oil sardine mince, except that the salt content in the salt-mince mixture was low (20%) as compared to threadfin bream mince (25%). According to Wojtowicz *et al.* (1977), the moisture content of salt minced fish should be 38% to 43%, when 'fast salting' technique is adopted, since at this level of moisture the undried product will be reasonably stable. Although the same technique has been adopted in the present study, the moisture content was around 50% and 51% for threadfin bream and oil sardine minces, respectively. The moisture contents could have been reduced to that level, provided we had centrifuged the samples. However, the moisture content was further brought down by oven-drying to 7.3% and 3.08% in case of threadfin bream mince and oil sardine shredded mince, respectively, while the moisture content of bricks made from threadfin bream was 18.8% for oven-dried samples (Table 2). The stability of the product at 38% to 43% moisture level, as stated by Wojtowicz *et al.* (1977), is rather doubtful for tropical countries like India. Even with a moisture content of 18.8% in threadfin bream bricks, the TMA-N and TVB-N values increased to 11.83 mg% and 140.98 mg%, respectively, during a storage period of three months (Table 5).

3.2 Rehydration Characteristics

The results of this study show that all three products, namely shreds and bricks of threadfin bream and shreds of oil sardine, possessed acceptable water binding capacity and texture after rehydration (Table 4). The shredded threadfin bream mince showed higher moisture content after rehydration and also the highest rehydration ratio, which may be attributed to the fact that it took the shortest time for drying compared to other samples. Oven-dried samples fared better with respect to rehydration capacity than sundried samples. While the oven-drying (50°C) took approximately 18-20 h for shredded threadfin bream mince, sundrying took 30-32 h and for drying the mince in the form of bricks approximately 30-32 h were required in the oven (50°C), whereas sundrying took 40 h or more, which was not desirable from the stand point of quality of the product. This was even more evident in the case of sundried oil sardine mince, which became rancid even before drying was completed. Oven-drying (50°C) of oil sardine mince in the form of bricks was found to take much more time, resulting in a rancid product; hence this product was prepared only in the form of shreds.

3.3 Chemical Changes During Storage

The results of the chemical changes during storage of salted mince of threadfin bream and oil sardine are shown in Table 5. As can be seen from the Table, the moisture content of salted threadfin bream bricks increased from an initial value of 18.80% to 31.39% over a period of three months, thereby indicating considerable increase in their moisture content. As a result of this, the salt content of the product decreased from 38.74% to 31.61%. The increase in moisture and decrease in salt content in the case of oil sardine shreds was only marginal. In the case of threadfin bream bricks, even though the TMA-N and TVB-N values showed a gradual increase during the period of storage, they remained well within the limits of acceptability (Connell, 1980). In the case of oil sardine shreds, the TMA-N value was nil throughout the storage period and the increase in TVB-N value was negligible. Valaan, Nair and Rao (1961) reported similar TVB-N values (151.2 mg% at the end of three months' storage) for salted and dried mackerel. The information on TMA-N and TVB-N values for salted and dried fish minces appears to be scanty. In the absence of detailed information on salted minces it is rather difficult to judge the product by these two parameters only, except to depend on the organoleptic evaluation. Even though the TBA values in respect of salted threadfin bream bricks also showed a gradual increase during storage they were within the acceptability limits (1-2 mmol/kg fat). However, in the case of salted mince made from oil sardines, the TBA values were higher and, therefore, the rancidity of the product was clearly evident during the taste panel tests.

The quality of protein in a product is frequently assessed by analysing its available lysine and its pepsin digestibility (Lovern, 1964). As can be seen from Table 7, threadfin bream had values above the minimum limits (92% pepsin digestibility and 6.5% available lysine) fixed for dried fish products (Heen and Kreuzer, 1962). After three months of storage, the pepsin digestibility decreased marginally to below the minimum limit in both samples. There was a similar decrease in lysine content in both samples, but it was above the minimum limit in the case of oil sardine shreds and slightly below the minimum in the case of threadfin bream bricks.

3.4 Microbiological Changes During Storage

The changes in microbial load over the storage period of three months were only marginal and well within the acceptable limits (Table 6). The increase in mould count (1.7×10^3 to 5×10^4 /g) and halophilic bacterial count (1.7×10^3 to 9.5×10^4 /g), which accounted for the major microflora of the salted fish products, were only a little more than one log during storage in the case of threadfin bream bricks. In the case of salted mince of oil sardine, there was no increase in the mould and halophilic bacterial counts throughout the storage period, presumably due to the very low moisture content of this product.

3.5 Acceptability

The acceptability studies showed that the salted minces prepared from threadfin bream, both in the form of bricks and shreds, were acceptable to all the panelists without any adverse remarks. However, the salted mince from oil sardine was not acceptable to the majority of panelists because of its distinct rancid flavour.

3.6 Economics

Since the oil sardine mince was not acceptable to the panelists, the cost of production was worked out only for the threadfin bream mince. The details of cost calculation are shown in Appendix 1. It works out to Rs 23.00/kg which is quite reasonable for a dried product and compares favourably with the prevailing cost of other dried traditional fish products.

4. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, 1975 D.C., AOAC
- APHA (American Public Health Association), Compendium of methods for the microbiological examination of foods. Washington, D.C., APHA
- Barequet, W.J., Dried salted fish - a rapid salting process - review. Bol.Inst.Tecnol.Aliment., 1974 38:13-37
- Beatty, S.A. and N.E. Gibbons, The measurement of spoilage in fish. J.Biol.Board Can., 3(1):77-91 1937
- Bligh, E.G., Salted minced fish (Brochure). Halifax, Nova Scotia, Environment Canada, Fish and Marine Services, 15 p.
- Bligh, E.G. and R. Duclos, Salting of minced fish. Fish by-catch... bonus from the sea. Report of a Technical Consultation on shrimp by-catch utilisation. Georgetown, Guyana, 27-30 October, 1981. Ottawa, International Development Research Centre (IDRC-1986):81-3
- Carpenter, K.J., Estimation of the available lysine in animal-protein foods. Biochem.J., 77:604-10 1960
- Connell, J.J., Control of fish quality. Farnham, Surrey, Fishing News (Books) Ltd., 222 p. 2nd ed. 1980
- Del Valle, F.R. and J.L. Gonzalez-Inigo, A quick salting process for fish. 2. Behaviour of different species of fish with respect to the process. Food Technol., 22:1135-8 1968
- Del Valle, F.R. and J.T.R. Nickerson, A quick salting process for fish. 1. Evaluation of the process. Food Technol., 22:1036-8 1968
- Del Valle, F.R., et al., Pilot plant production and large-scale acceptance trials with quick salted fish cakes. J.Food Sci., 38:246-50 1973
- _____, Proximate analysis, protein quality and microbial counts of quick-salted freshly made and stored fish cakes. J.Food Sci., 41:975-6 1976
- FAO, The prevention of losses in cured fish. FAO Fish.Tech.Pap., (219):87 p. Issued also in French 1981
- Heen, E. and R. Kreuzer, Fish in nutrition. London, Fishing News (Books) Ltd., for FAO, 445 p. 1962
- Loescheke, H.W., von, Rehydration of dehydrated foods; some analytical methods. In Drying and dehydration of Foods. New York, Reinhold Publishing Corp., p. 284, 2nd ed.
- Lovern, J.A., Pepsin digestibility: as an index of quality in fish. Fish.News.Int., 3:209-10 1964
- Mandelsohn, J.M., Rapid techniques for salt curing fish - a review. J.Food Sci., 39:125-7 1974
- Revenkar, G.D., A.K. Naider and B.L. Baliga, Frozen fish curry and its storage stability. Indian Food Pack., 33(3):8-10 1979

- Velsan, A.P., M.R. Nair and S.V.S. Rao, Propionic acid as a preservative for cured fish products.
1961 J.Sci.Food Res., 20(9):331-7
- Wojtowicz, M.B., M.G. Fierheller and L.W. Reiger, Observations on salting minced leao fish. Paper
1975 presented at the 20th Annual Conference of AFT, Washington, D.C. (mimeo)
- _____, Making "instant" salt minced fish. Circ.Fish.Oceans, Halifax, Can., (68):13 p.
1978
- Wojtowicz, M.B., *et al.*, A technique for salting lean minced fish. Tech.Nsp.Fish.Mar.Serv.Can.,
1977 (731):15 p.
- Young, R.H., *et al.*, Development and acceptability testing of modified salted fish product prepared
1979 from shrimp by-catch. J.Food Technol., 14(5):509-19
- Yu, T.C. and R.O. Sinnhuber, 2-Thiobarbituric acid method for the measurement of rancidity in
1957 fishery products. Food Technol., 11(2):104-8

Table 1

The percentage yield of dressed, pickled meat and salted minces

Material	Percentage (%)	
	Oil sardine	Threadfin bream
Dressed fish in relation to whole fish	57.3	60.5
Pickled meat in relation to whole fish	37.3	36.0
Salted mince in relation to whole fish	9.7	13.5
Salted mince in relation to pickled meat	26.1	37.5
Salted mince in relation to dressed fish	16.9	22.3

Table 2

Percentage of proximate composition of pickled meat and final products

Sample	Oil sardine			Threadfin bream			
	Pickled meat	Water-bloached meat	Salted, dried mince (shreds)	Pickled meat	Salted and dried bricks		Salted dried shreds
					Freshly made	After 3 months	
Moisture	67.4	72.45	3.08	81.19	18.8	31.39	7.30
Crude protein	18.74	20.90	49.3	14.4	41.8	37.0	47.0
Crude fat	11.7	4.4	9.8	0.74	1.0	0.9	1.0
Ash	1.6	1.53	-	2.1	-	-	-
Salt (NaCl)	-	-	37.72	-	38.7	31.61	44.08
			38.57				41.98

Table 3

Composition of mince of threadfin bream (TFB) and oil sardine (OS) in relation to salting ratio

Salt to fish mince ratio	Concentration of salt (%) in mince-salt mixture	(1) Brine removed TFB		OS		Composition (%)				Fish solids	Water	OS	Fish solids	Characteristics	Remarks
		TFB	OS	TFB	OS	Salt	Water	Fish solids	Salt	Water	OS	Fish solids	OS		
1:4	20	50	30	21.5	60	18	20	65	13	Net, sticky	13	Net, sticky	13	Net, sticky	Incomplete salting
1:3	25	54	52	29	50	21	28	51	16	Dry, easy to separate brine	16	Dry, easy to separate brine	16	Dry, easy to separate brine	Complete/ optimum salting
1:2	33.3	52	50	38	45	16	38.5	47	12	Dry, easy to separate brine, excess waste of salt found in the mince and cake	12	Dry, easy to separate brine, excess waste of salt found in the mince and cake	12	Dry, easy to separate brine, excess waste of salt found in the mince and cake	Over salt- ing.

Table 4

Rehydration ratios and moisture content of salted and dried minces

Sample	Rehydration ratios			(%) Moisture of salted and dried samples	(%) Moisture of rehydrated samples
	5	10	20		
Threadfin bream bricks	1.16	1.361	1.554	20.65	62.74
Threadfin bream shreds	1.43	1.92	2.17	7.55	68.61
Oil sardine shreds	1.38	1.69	2.05	4.62	63.87

Table 5
Changes in chemical characteristics of salted and dried minces during storage

Storage period (months)	Brickets of threadfin breams				Shreds of oil sardine			
	Moisture (%)	Salt (%)	TMA (mg %)	TUN (mg %)	TBA value (No. of moles of malonaldehyde/1000g sample)	Moisture (%)	Salt (%)	TBA value (No. of moles of malonaldehyde/1000g sample)
0	19.80	38.74	3.71	29.68	0.773	3.09	37.72	Nil
1	32.00	30.74	11.13	33.39	1.273	3.73	37.9	Nil
2	31.63	31.34	14.84	77.91	1.409	4.58	39.75	Nil
3	31.39	31.61	11.13	140.98	0.999	4.62	38.57	Nil
								1.045
								1.341
								4.272
								2.386

Table 6
Changes in microbiological characteristics of salted and dried minces during storage

Storage period (months)	Brickets of threadfin breams			Shreds of oil sardine		
	Mould count (No./g)	Malophilic bacteria (No./g)		Mould count (No./g)	Malophilic bacteria (No./g)	
0	1.70×10^3	1.70×10^3		3.10×10^3		30 in 10^{-1} dilution
1	8.10×10^3	3.80×10^4		"		"
2	5.60×10^4	4.70×10^4		"		"
3	5.00×10^4	9.50×10^4		1.40×10^3		"

Table 7
Protein quality of salted and dried minces

Sample	Pepsin digestibility (% protein)		Available lysine (% protein)	
	Freshly made	After 3 months	Freshly made	After 3 months
Oil sardine shreds Threadfin bream brickets	93.00	89.5	7.16	6.77
	93.25	89.75	6.64	6.28

APPENDIX 1

Cost Calculation for Production of Salted Threadfin Bream Minca
on a Pilot Plant Scale

Number of working days: 200/year
Capacity of plant: 500 kg of fish/day

	<u>Qty</u>	<u>Rs</u>
A. Details of capital investment		
1. Building (processing hall)		100 000.00
B. Machineries and equipment		
1. Meat picking machina	1	35 000.00
2. Meat mincer	1	25 000.00
3. Hand-operated screw press	1	5 000.00
4. Built-in-room type dryer	1	75 000.00
5. Fish-drassing tables	3	2 500.00
6. Salting vats	5	2 500.00
7. Miscellaneous items like trays, knives, buckets, balance, wooden planks, sieves, etc.		10 000.00
8. Installation and organization cost		10 000.00
Total		<u>264 000.00</u>
C. Details of working expenses/day - cost/ton		
1. Fish at Rs 2/kg - Rs 2 000	500 kg	1 000.00
2. Powdered salt at Rs 1/kg - Rs 1 000	65 kg	65.00
3. Labour charges at Rs 15/day/person for 3 labourers		45.00
4. Packing cost at Rs 0.5/kg for 65 kg		32.50
5. Staff (supervisory) at Rs 20/day		20.00
Production of salted minca/day = 65 kg		<u>1 162.50</u>
Production cost for 13 t of salted minca/year of 200 working days		<u>232 500.00</u>
D. Details of other expenses		
1. Depreciation on building (at 5% on Rs 100 000)		5 000.00
2. Depreciation on machineries (meat picker, mincer and press at 10% on Rs 65 000)		6 500.00
3. Depreciation on dryer, tables and vats (at 20% on Rs 80 000)		16 000.00
4. Depreciation on miscellaneous items (at 10% on Rs 10 000)		1 000.00
5. Interest on capital investment (at 12% on Rs 265 000)		31 800.00
6. Interest on working capital (at 12% on Rs 35 000 expenses for about 30 days)		4 200.00
7. Electricity charges		10 000.00
Total		<u>74 500.00</u>
Total gross expenses/year (C + D)		307 000.00
Less receipts from sales of dried fish wastes (10 t) at Rs 1/kg		10 000.00
Total net expenses/year for production on 13 t of salted mince		<u>297 000.00</u>
Cost of production/kilogramme of salted minca		22.84
or say		<u>23.00</u>

HISTAMINE CONTENT OF SMOKE-DRIED FRIGATE MACKEREL (*Aurio thazard* L.)

by

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ABSTRACT

The study deals with the comparative chemical and microbiel analysis of both experimental and commercially available smoke-dried frigate mackerel. The chemical analyses included: histamine content, total volatile base-nitrogen (TVB-N), salt content, pH, water activity (A_w) and proximate analyses. Total plate count (TPC) was the only microbial examination conducted.

Analyses revealed that both commercial and experimental smoke-dried frigate mackerel have safe levels of histamine that range from 3.90 mg % to 5.90 mg %. Method of preparation, e.g., brining and drying time, have a significant effect on the histamine content of the product.

Water activity (A_w), moisture content and NaCl content significantly affect the levels of histamine in the product while pH and TVB-N do not.

Microbial examination showed that commercial hot-smoked frigate mackerel has a higher microbial load than experimentally prepared product. However, the microbial load of commercial fish is not responsible for histamine formation as indicated by the low levels of histamine in the product.

1. INTRODUCTION

The Philippines is the largest tuna producer in Southeast Asia (Aprieto, 1980) and has a great need to improve processing technology, identify and strengthen markets and develop innovative product forms that would be acceptable and competitive in both foreign and local markets. Tuna-like species, specially frigate mackerel (*Aurio thazard* Lacapède) which is still underutilized (Elizalde, 1982), can be prepared in processed forms which are commercially accepted, such as salted fish flakes, fish chips, fish sausa, fish pasta, fish sausage and fish protein concentrats.

Apart from the products mentioned above, a hot-smoked product from frigate mackerel has been locally produced and marketed in rural coastal areas. However, consumption of tuna in processed form is comparatively small, though gradually increasing. Lack of exposure to the products and some prevailing taboos, such as the species being regarded as poisonous in some remote villages, inhibit consumer attitudes (Elizalde, 1982). Hence, histamine content in smoke-dried mackerel needs to be studied.

2. REVIEW OF RELATED LITERATURE

2.1 Exploitation and Production Potential of Tuna Industry in the Philippines

The Philippine tuna fishery has become the single biggest fishery industry in the country during the last five years (Aprieto, 1980). The production of tuna and tuna-like species over the last five years (1976-80) has increased with an average growth rate of 16.5%. From 1978, commercial production of tuna registered a dramatic 65% increase. In addition, experts have projected potential yield of oceanic waters at 20 000 t in 1980, progressing to 107 000 t by 1990. These recent developments with the escalating demand for fish products underscore the need for the local tuna industry to improve its market (Elizalde, 1982).

The four commercial species of tuna are: skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacore*), eastern little tuna (*Euthynnus yafu*) and frigate mackerel (*Aurio thazard*). Among these species, yellowfin and skipjack are the most abundant, and have big demand in the export market (Aprieto, 1982). Ninety-two percent of the country's total exported frozen tuna in 1978 and 1979 went to the US and Japan (Elizalde, 1982). Industry watchers, however, observe that small tunas are abundant in most parts of the country and are still underutilized.

Because of the low appeal of tuna to local consumers, innovations in product preparation need to be directed toward changing the natural characteristics of the fish to more marketable forms (Elizalde, 1982).

2.2 Smoke-Dried Frigate Mackerels and Similar Products

A great variety of cured fish product has evolved through the centuries, particularly in the developing countries. "Katsuobushi", for instance, which is a unique dried product in Japan, is prepared using tuna as raw material (Tanikawa, 1969). Waterman (1976) described the product as a hard dried skipjack tuna, made by cutting the fish longitudinally into four, removing bones, boiling, moulding and drying. The product is shaped and defatted by the controlled enzymic action of moulds. However, the process of making "Katsuobushi" in the Philippines is modified by eliminating the defatting steps (NSDB, 1980).

Drying "katsuobushi" reduces moisture content, thus creating an unfavourable environment for the growth of yeasts and moulds (Hall, 1957 as cited by Milla, 1982). Consumers have low preference for "katsuobushi" because the product has low moisture content, or is too dry (Kapsalis, 1975). Hence intermediate moisture food (IMF) has been developed for human consumption, as well as pet food. IMF can be eaten without rehydration, and is very stable even without refrigeration or thermal processing. Thus, IMF is defined as a product moist enough to be ready to eat and yet dry enough to have a stable shelf life. The principles involved in developing IMF, e.g., pet food, can be applied to the formulation of IMF for human consumption (Kaplow, 1970).

2.3 Spoilage Behaviour of Intermediate Moisture Foods

2.3.1 Chemical spoilage

IMF and dehydrated food exhibit similar chemical changes (Brockman, 1970). Spoilage of such products may be due to rancidity, browning and other reactions (Doe *et al.*, 1981), specifically decarboxylation of histidine to form histamine because of the nature of the raw material used, frigate mackerel, which is known as "scombroid poisoning". Grantham (1981) cited additional problems to be overcome in the development of IMF, such as the negative flavour attributes of the humectants, oxidative rancidity, enzymatic and non-enzymic browning. Brockman (1970) noted that IMF is more susceptible to Maillard reactions than dehydrated food, but less susceptible to fat degradation. Non-enzymic browning is often accompanied by the development of bitter flavours in food (Pangan, 1976). On the other hand, enzymatic reaction (in the presence of oxygen) brings about off-flavour development and loss of nutrients in the food (Meyer, 1960 cited by Pangan, 1976). Aside from these changes, total volatile bases-nitrogen (TVB-N) might also play an important role during storage (Sunaydeng, 1978).

2.3.2 Microbial spoilage

Microbial growth is very dependant on temperature and water activity (Troller and Christian, 1978). Spoilage in dried fish is attributed to micrococci and Gram positive rods (Liston, 1980). Aside from these micro-organisms, dun mould *Mullerula sebi* (Syn. *Sporodroma opusum*) is commonly found on dried fish in tropical countries (Waterman, 1976; Troller and Christian, 1978). Liston (1980) noted the prevalence of *Aspergillus* spp., *Cooperia* spp. and *Penicillium* spp. on dried fish. It is most likely that IMF will have the same microflora because of its water activity (A_w) which is slightly higher than dried products.

2.3.3 Materials and methods

2.3.3.1 Materials: Frigate mackerel (*Aurie thazard* L.), locally known as "tulingan", was used for the preparation of smoke-dried product. The fish were taken from Navotas Fish Landing and transported to UPWCP, DPPT Laboratory with proper icing.

2.3.3.2 Methodology: The study was divided into three parts: Part I was concentrated on the preparation of smoke-dried frigate mackerel; Part II mainly dealt on the physico-chemical analyses of commercial and experimental smoke-dried product; and Part III basically involved microbial load examination of both commercial and experimental smoke-dried product.

Part I - Preparation of Smoke-Dried Frigate Mackerel

Iced frigate mackerel was washed, eviscerated and soaked in saturated brine solution for varying times. After brining, the fish were boiled for 10 min using weak brine solution. The boiled fish were smoked for 3 h at a temperature range of 60-70°C. Smoked frigate mackerel were dried for another 3 h at a temperature that varied from 50° to 60°C. Finished product should have a moisture content that ranges from 35% to 45%. Flow sheet diagram of the process is shown in Chart 1.

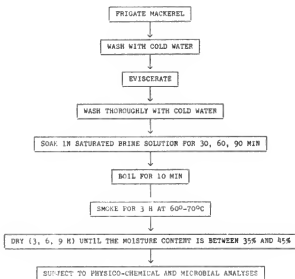


Chart 1 Scheme for the preparation of smoke-dried frigate mackerel

Part II - Physico-Chemical Analysis

- (i) Water Activity Measurement: The methods of Doa *et al.* (1981) and Lupin, Boari and Maschiar (1981) were used in the determination of water activity of smoke-dried frigate mackerel.
- (ii) pH: The pH of the product was measured on 2 g of the sample homogenized with 20 ml of distilled water for 1 min. The pH was determined immediately after the preparation of the sample.
- (iii) Salt content: The Volhard method (cited by Nieto, 1983) was employed in salt content determination.
- (iv) Histamine Content Determination: Levels of histamine in the product were determined by the fluorometric method.
- (v) TVB-N: The Conway method of determining TVB-N was used in the study.
- (vi) Proximate Analysis: The Kjeldahl method was employed in the determination of protein. The method of Bligh and Dyer was used for fat analysis, and AOAC (1970) method for ash content. The moisture was measured using an Infrared Moisture Tester (Ohaus).

Part III - Microbial Examination and Sensory Evaluation of Smoke-Dried Frigate Mackerel

Total Plate Count (TPC) was done before and during storage. The plates were incubated at ambient temperature for 18-24 h. Smoke-dried frigate mackerel was also subjected to sensory evaluation. The panel consisted of six members and the analysis was limited to descriptive evaluation of the product, e.g., texture, flavour, odour and colour.

3. RESULTS AND DISCUSSIONS

Commercial samples of hot-smoked frigate mackerel have low levels of histamine that range from 5.30 to 5.90 mg/100 g, as shown in Table 1; while experimentally prepared smoke-dried frigate mackerel has a range of 3.90 to 5.12 mg I (Table 2). Length of soaking in saturated brine significantly reduced the amount of histamine. Table 2 shows that the longer the soaking period in saturated brine the higher the salt content. Salt inhibits microbial growth, hence it may reduce the amount of histamine.

Another important factor that contributed to low histamine content is the water activity (a_w); Doe et al. (1981) and Lupin, Boeri and Maschiar (1981) methods of determining water activity (a_w) do not give significant differences, as shown in Tables 1, 2 and 3. Salt uptake and water loss are influenced by the fat content of the fish, thickness of flesh, freshness and chemical purity of the curing salt (Desrosier, 1970). However, considering that frigate mackerel is classified as a medium fat fish (Arroyo, 1974), fat content might have little effect on salt uptake and water loss. Low moisture content reduces microbial activity, hence a low amount of histamine is formed in the product.

Total Plate Count (TPC) revealed that commercial hot-smoked frigate mackerel have higher microbial loads than the experimentally prepared smoke-dried frigate mackerel. However, histamine content of both products are within the safe level since the maximum allowable level in most fish products is around 10 mg histamine/100 g of fish (Arnold and Brown, 1978). The result simply implies that microbial activity, which is responsible for the formation of histamine, is greatly affected by the process involved in the production of smoke-dried product, e.g., soaking in saturated brine, boiling, smoking and drying. In the process, micro-organisms which are not salt tolerant, not heat-loving and do not like low moisture are eliminated. Hence, mesophiles, which are responsible for decarboxylation of histidine to histamine, have a very small chance of surviving during processing. High microbial load of commercial hot-smoked frigate mackerel can be attributed to post-process contamination.

The amount of TVB-N which varies from 23.50 to 55.10 mg % of hot-smoked frigate mackerel is higher than that of experimentally prepared product, with TVB-N range of 12.40 to 20.90 mg %. Moisture level has no significant effect on the amount of TVB-N, as shown in Table 3. TVB-N in the processed product, which is 29.31 mg %, is higher than fresh fish with 9.79 mg % (Table 4) because TVB-N was found to increase to 33 mg % after drying (Bello and Pigott, 1979). High amount of TVB-N in commercial hot-smoked frigate mackerel can be attributed to high microbial load which might be due to post-process contamination. It should be noted, however, that TVB-N, like pH, does not contribute significantly to the formation of histamine in smoke-dried frigate mackerel.

Histamine, TVB-N, protein, ash and salt content were found to be higher in smoke-dried product than in fresh fish while a reduction in pH and water activity was noted (Table 4). Reduction in pH can be attributed to heat treatment and addition of potassium sorbate (Bello and Pigott, 1979), while dehydration resulted to low water activity (a_w).

Low levels of moisture obtained after 9-h drying reduces microbial load and amount of TVB-N, however, low amount of moisture affects the quality of the product.

Proximate analyses revealed that the product has a high protein content; protein content ranges from 22.94% to 38.81%, while fat content varies from 3.04% to 4.81%.

Sensory evaluation indicated that the panelists preferred an intermediate moisture product with 60% moisture and 2.19% salt (Tables 2 and 3).

4. CONCLUSION AND RECOMMENDATION

Frigate mackerel, a species underutilized in the Philippines, is commonly processed in rural coastal areas into a hot-smoked product. High bacterial count, TVB-N content and histamine level of the hot-smoked product can be due to its high water activity (a_w of 0.99). The water activity can be reduced by the application of salting, boiling, smoking and drying procedures.

Thus, smoke-dried frigate mackerel which was soaked in saturated brine solution for 30 min and dried for 9 h after smoking for 3 h was developed. The product has lower level of histamine, bacterial count and TVB-N content. This is probably due to its lower water activity (a_w of 0.97).

On the public health aspects, detection and enumeration of some pathogenic organisms, particularly *Salmonella* and *Staphylococcus aureus*, should be investigated.

5. REFERENCES

- Aprieto, V.L., Philippine tuna fisheries. Resources and industry. A special report. Fish.Res.J. Philip., 5(1):55-66
1980
_____. Philippine tuna fishery management. Fish.Res.J.Philip., 7(1)
1982
Arnold, S.W. and W.D. Brown, Histamine (?) toxicity from fish products. Adv.Food Res., 24:113-54
1978

- Arroyo, P.T., The science of Philippine foods. Araneta Center, Quezon City, Philippines, Abaniko Enterprises. 1st ed.
1974
- AOAC (Association of Official Agricultural Chemists), Official methods of analysis. Washington, D.C., AOAC
1970
- Bello, R.A. and G.M. Pigott, A new approach to utilizing minced fish flesh in dried products.
1979 J.Food Sci., 44(2):335-8
- Brockmann, M.C., Development of intermediate moisture foods for military use. Food Technol., 24
1970
- Desrosier, N.W., The technology of food preservation. Westport, Connecticut, AVI Publications,
1970 3rd ed. 493 p.
- Doe, P.E., Spoilage of dried fish: the need for more data on water activity and temperature
1983 effects on spoilage organisms. FAO Fish.Rep., (279)Suppl.: 209-13
- Doe, P.E., et al., Isohalic sorption isotherms. 1. Determination of dried salted cod (*Gadus morhua*).
1981 J.Food Technol., 17:125-34
- Elizalde, L.P., Prospects for tuna product development. Fish.Today, 4(1):36-40
1982
- Graham, G.J., Minced fish technology: a review. FAO Fish.Tech.Pap., (216):72 p. Issued also
1981 in French and Spanish
- Heise, R. and K. Eichner, Moisture content and shelflife. Food Manuf., 46(5):53-8
1971
- Kaplow, M., Commercial development of IMF. Food Technol., 24:889-93
1970
- Kaplanis, J.G., The influence of water on textural parameters in foods at intermediate moisture
1973 levels. In: Water relation of foods, edited by R.B. Duckworth. London, Academic Press
- Labuza, T.P., Sorption phenomena in foods. Food Technol., 22:63
1968
- Liston, J., Microbiology in fishery science. In: Advances in fish science and technology, edited
1980 by J.J.-Connell. Farnham, Surrey, Fishing News (Books) Ltd., pp. 138-37
- Lupin, H.M., R.L. Boeri and S.M. Maschiar, Water activity and salt content relationship in moist
1981 salted fish products. Food Technol., 16:31-8
- Milla, A., Standardization of drying temperature and time using the AFOS Mechanical kiln. Special
1982 problem. College of Fisheries, University of the Philippines in the Visayas, Diliman, Quezon City, Philippines
- Nieto, M.B., Factors affecting the ripening and flavor of Bagoong alamang (shrimp paste). Graduate
1983 thesis. College of Home Economics, University of the Philippines, Diliman, Quezon City, Philippines
- NSDB (National Science Development Board), Philippines handbook on fish processing technology.
1980 Bicutan, Taguig, Metropolitan Manila, Philippines, NSDB
- Pangan, A.C., Storage and packaging of ready-to-fry squid (*Loligo pealii*) Kroebeck. Undergraduate
1976 thesis. College of Home Economics, University of the Philippines, Diliman, Quezon City, Philippines
- Sumaydeng, C.L., Storage stability of dried, seasoned squid (*Loligo* sp.). Undergraduate thesis.
1978 College of Fisheries, University of the Philippines, Diliman, Quezon City, Philippines
- Tanikawa, E., Marine products of Japan. Tokyo, Koseishako-seikaku Co.
1969
- Troller, J.A. and J.H.B. Christian, Water activity in foods. London, Academic Press
1978
- Waterman, J.J., The production of dried fish. FAO Fish.Tech.Pap., (160):52 p. Issued also in
1976 French and Spanish

Table 1

Characteristics of a commercial sample
of a smoke-dried frigate mackerel (*Aurlo thazard*, L.)

Parameters	Commercial samples		
	1	2	3
Histamine (mg %) ^{a/}	5.90	5.30	5.50
TPC (log)	7.70	7.98	7.09
TVB-N (mg %)	32.30	55.00	23.50
pH	5.29	5.26	5.26
A _w (Doe et al., 1981)	0.98	0.98	0.98
(Lupin, Boeri and Maachiar, 1981)	0.99	0.99	0.99
% NaCl	0.95	0.89	0.72
% moisture	58.41	60.40	54.64
% fat	4.55	4.55	3.69
% protein	24.55	22.94	27.32

^{a/} 100 g wet weight

Table 2

Characteristics of local katsuoobushi from frigate mackerel
with varying length of brining time in saturated brine solution

Parameters	Brining time (min)			
	0	30	60	90
Histamine (mg %) ^{a/}	4.80	5.12	4.82	3.90
TPC (log)	3.43	2.58	2.86	3.15
TVB-N (mg %)	20.90	12.40	16.90	18.90
pH	5.54	5.62	5.53	5.60
A _w (Doe et al., 1981)	0.985	0.980	0.975	0.97
(Lupin, Boeri and Maachiar, 1981)	0.988	0.975	0.970	0.964
% NaCl	1.19	2.19	2.60	3.06
% moisture	59.85	58.58	58.39	57.66
% fat	1.61	2.37	2.63	2.63
General acceptability	5.34	6.85	6.25	6.73

^{a/} 100 g wet weight

Table 3

Characteristics of smoke-dried frigate mackerel
with varying moisture levels

Parameters	Moisture levels (%)		
	60	55	50
Histamine (mg %) ^{a/}	4.85	5.05	4.75
TPC (log)	4.80	5.10	4.17
TVB-N (mg %)	19.20	20.80	22.20
pH	5.70	5.68	5.61
A _w (Doe <i>et al.</i> , 1981)	0.98	0.97	0.965
(Lupin, Boeri and Maschiar, 1981)	0.974	0.966	0.959
% NaCl	2.20	2.55	2.77
% moisture	57.02	50.16	46.33
% fat	1.78	1.85	1.88
General acceptability	6.52	5.85	6.40

a/ 100 g wet weight

Table 4

Physico-chemical characteristics of raw fish^{a/}
and a smoke-dried frigate mackerel

Determination ^{b/}	Fresh	Processed
% moisture	71.83	49.84
% salt	1.06	1.93
% crude fat	1.90	3.04
% crude protein	19.92	38.81
% ash	5.83	6.13
% potassium sorbate		0.08
A _w ^{c/}		0.956
pH	5.51	5.47
TVB-N (mg %)	9.79	29.31
Histamine (mg %)	4.16	5.20

a/ Fresh fish was used in this study

b/ Mean of two trials

c/ A_w value analyser model (Lufft) 5803

TOXIC AMINES IN FISH

by

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ABSTRACT

High levels of histamine in certain pelagic fish have been implicated in cases of food poisoning (so called scombroid poisoning), a well-known problem during the handling of mackerel and tuna. Six commercially important fish species from Ecuador were examined with respect to histamine formation during spoilage. Three species were found to have significant histamine levels after 8-10 h spoilage at ambient temperatures with rates of accumulation varying widely from species to species. It was concluded that there was a serious risk that for some species potentially toxic levels of histamine may occur in fish which would otherwise be regarded as acceptable, hence scombroid type poisoning may be significant in many tropical countries.

1. INTRODUCTION

Scombroid poisoning, associated with the consumption of fish containing high levels of histamine, is a well documented problem with pelagic fish such as mackerel and tuna (Arnold and Brown, 1978). It is due to the high levels of free histidine (a non-toxic amino acid) being converted to the biologically active histamine by bacteria which invade the fish flesh as it spoils. Symptoms of acute poisoning include severe headache, sore throat, facial flushing, shock, vomiting and diarrhoea, and very rarely death. Although the exact characteristics of the toxicity appear to be complex end may also involve amines other than histamine, fish implicated in outbreaks of food poisoning of this type have been found to contain elevated histamine levels, usually in excess of 100 mg per 100 g fish (mg %).

The United States Food and Drug Administration have produced a Compliance Policy Guide for Albacore, Skipjack and Yellowfin Tuna (United States Food and Drug Administration, 1980) which specifies that histamine levels of over 50 mg % should be regarded as a danger to health while a batch would be considered to have decomposed if more than two subsamples (of 24) have levels of over 20 mg %. A lower level of 10 mg % is considered by some experts to be a suitable maximum limit (Arnold and Brown, 1978). Apart from public health considerations, histamine levels have become of commercial importance as exporters of frozen and canned pelagic fish must ensure that their products conform to the standards set by their customers.

Ecuador has a developing export trade in frozen fish, the USA being a major market. A recent study on a major exported species, dorado (*Coryphaena hippurus*), revealed that poor handling after catching could lead to greatly elevated histamine levels. Significantly fish with histamine levels above 10 mg % were still regarded as acceptable by a taste panel (Sostock, Barratt and Cambo, in preparation). Clearly it would be advantageous to know which species were susceptible to histamine formation so that improved handling procedures can be introduced where required. Accordingly samples of fish of six different species of commercial importance to Ecuador were sent to the Tropical Development and Research Institute, London, for analysis after they had been stored at ambient Ecuadorian temperatures for various times.

2. MATERIALS

The species studied were as shown below:

Code	Local Name	Scientific Name
A	Dama	<i>Hemicaranx leucurus</i>
B	Pompano	<i>Trachinotus patenensis</i>
C	Pompano	<i>Trachinotus sp.</i>
D	Caballito	<i>Caranx caballus</i>
E	Huaycipe	<i>Seriola rivoliana</i>
F	Huaycipe	<i>Seriola peruviana</i>

3. METHODS

Several fish of the various species were obtained immediately after capture and iced prior to commencing the storage trial. Hence at the start of the storage trial all the fish were in a very fresh state. Fish were removed from the ice and stored at ambient temperatures (25°-29°C) in the whole gutted form. Samples were taken at intervals and frozen immediately. Storage was continued until deterioration was very advanced (strong faecal odours). The frozen samples were transported to TDRI, London, in an insulated box and were still frozen on arrival. They were stored at -30°C for less than 1 month and were thawed immediately before analysis for histamine.

Histamine was assayed using a Baird Atomic Fluoriscord Spectrofluorimeter by the method of Taylor, Lieber and Leatherwood (1978), wave-lengths calibrated using quinine sulphate (Taylor and Lieber, 1977). This method was found to give a linear relationship between fluorescence and histamine over the range 0-1 000 mg %. Fluorescence was found to decrease in the light source, a 5-min exposure reducing fluorescence by 50%. In view of this the instrument was calibrated by setting the gain controls to give a gauge reading of about 85% deflection using a standard histamine sample which was used only for this purpose. Calibration was completed by reading additional (identical) histamine standard solutions. All readings were taken as quickly as possible in order to minimize the length of exposure of the samples to the light source.

4. RESULTS

The results of the assays, given in Table 1, indicated that species A, B and C did not accumulate histamine; these experiments were discontinued. All of the available samples of species D, E and F were analysed for histamine, the results being presented in Figures 1, 2 and 3 respectively. It can be seen that these three species started accumulating histamine after about 8 h at ambient temperatures and that levels rose rapidly after this time. The rate and extent of accumulation varied markedly from species to species, species D being the fastest and greatest accumulator followed by F then E.

Table 1

Accumulation of histamine at ambient temperatures, species A (*Hemicorax atrinatus*), B (*Trachinotus patiens*) and C (*Trachinotus* sp.)

Fish species (code)	Sample No.	Length of storage at ambient temperatures (h)	% histamine (mg %)
A	1	13	0.6
A	2	13	0.6
A	3	13	1.0
B	1	13	1.0
B	2	13	2.9
C	1	13	1.0
C	2	20.3	1.4

Differences were observed between individual fish of the same species (for example species F at 13 h storage, fish 1: 30.2 mg %, fish 2: 176.6 mg %), however the general trend was in all cases similar.

5. DISCUSSIONS AND CONCLUSIONS

The results presented here clearly demonstrate that there are marked and extreme species specific differences in the tendency for fish to accumulate histamine during storage at ambient tropical temperatures. Bostock, Barratt and Camba (in preparation) have described how *Coryphaena hippurus* has been implicated in outbreaks of food poisoning due to the inadequate nature of fish handling in parts of Ecuador. The results of this study suggest that similar problems will probably be encountered with species E and F (*Seriola lalandi* and *Seriola peruviana*) while even greater problems can be anticipated with species D (*Caranx caballus*) which can have unacceptably high histamine levels after only 8 h (i.e., before the fish would normally be regarded as spoilt). In contrast histamine formation should not be a problem with species A, B and C.

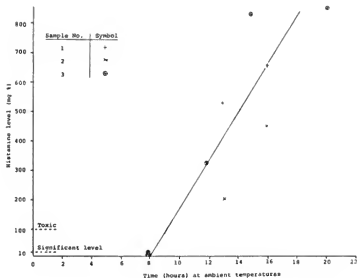


Figure 1 Accumulation of histamine at ambient temperatures, species D (*Trachinotus* sp.)

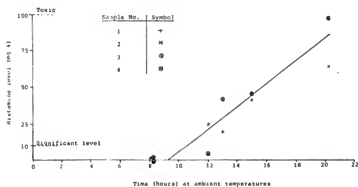


Figure 2 Accumulation of histamine at ambient temperatures, species E (*Seriola colburni*)

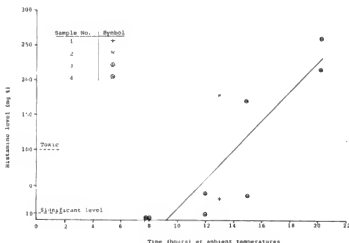


Figure 3 Accumulation of histamine at ambient temperatures, species F (*Seriola peruviana*)

These differences presumably relate in part to the free histidine levels found in the fish flesh. All six of the species studied during this work are from the family Carangidae, biologically far removed from the family Scombridae or Coryphaenidae (to which *Coryphaena hippurus* belongs). At present it is unclear why there should be such marked differences between these species but the data do strongly indicate that histamine formation is a more widespread hazard than previously thought. Handling conditions will determine how much of the histidine is converted to histamine. Hence there is a need to identify which tropical species have the potential for accumulating histamine, to devise satisfactory handling and quality control procedures to prevent potentially toxic fish reaching the consumer and to further investigate the quality of commercially available fish to identify where improvements are needed.

REFERENCES

- Arnold, S.H. and W.D. Brown, Histamine (?) toxicity from fish products. *Adv. Food Res.*, 24:113-5 1978
- Bostock, T., A. Barratt and N. Canba, A study of histamine in dorado (mahimahi; *Coryphaena hippurus*) and its relationship with product quality from the Ecuadorian artisanal fishery (in preparation)
- Taylor, S.L. and E.R. Lieber, Specificity and sensitivity of seven histamine detection methods. 1977 *J. Food Sci.*, 42:1584-6
- Taylor, S.L., E.R. Lieber and M. Leatherwood, A simplified method for histamine analysis of food. 1978 *J. Food Sci.*, 43:247-50
- United States Food and Drug Administration, Compliance policy guide, No. 7108.24. Washington, D.C., 1980 Food and Drug Administration

HISTAMINE FORMATION BY BACTERIA
ISOLATED FROM BULLET MACKEREL (*Auris rochet* Risso)

by

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ABSTRACT

Fresh bullet mackerel (*Auris rochet* Risso) was allowed to spoil at ambient temperature (30-32°C) for 24-48 h. Bacterial isolates were obtained from the standard plate counts at 37°C after 48 h. Ninety five percent (95%) of the isolates were found to produce histamine in culture media which contained histidine, and 70% were identified as *Proteus* spp. Histamine production in fish infusion broth by *Proteus vulgaris*, *Proteus morganii* and *Proteus mirabilis* was determined at 37, 20, 7 and 1°C. At 37 and 20°C *Proteus morganii* formed the highest concentrations of histamine (600-700 mg/l) within 1 day, and levels decreased to 200-350 mg/l after 7 days. No histamine was formed at 1°C, indicating that the rapid chilling of mackerel may suppress histamine formation.

1. INTRODUCTION

The occurrence of histamine toxicity, often referred to as scombroid poisoning, is well documented (Taylor, 1983; Arnold and Brown, 1978). Scombroid fish muscle contains high levels of free histidine which is decarboxylated to histamine by bacterial enzymes. Many different species of bacteria have been reported to be responsible for histamine production in fish and fishery products, both at chilled and ambient temperature (Okuzumi, Okuda and Awano, 1981; Yoshinaga and Frank, 1982; Taylor, Lieber and Leatherwood, 1978; Okuzumi, Okuda and Awano, 1982). In commercial practice, especially with low-income fishermen, fish are not iced or inadequately iced after catching. Storage at elevated temperature leads to prolific microbial growth and a rapid deterioration in quality. It is common to find scombroid fish, such as bullet mackerel (*Auris rochet* Risso) in retail markets exposed to ambient temperatures for long periods which are ideal conditions for the formation of toxic levels of histamine (Frank, Yoshinaga and Nip, 1981).

The objective of this study was to identify the bacteria responsible for histamine production during the spoilage of bullet mackerel at ambient temperatures, and to measure histamine formation by these bacteria at chilled and elevated storage temperatures.

2. MATERIALS AND METHODS

2.1 Sampling

Medium sized fresh fish were obtained from a local market and stored in sterile plastic bags at ambient temperature (28-32°C) for 24-48 h.

2.2 Microbiological Analysis

A 15 g sample from the dorsal section of two fish was taken for each trial and standard plate counts at 37°C carried out on iron agar (Jensen and Schultz, 1980). A total of 40 colonies were randomly isolated and purified on nutrient agar. Bacteria were identified according to the methods of Cowan (1974) and MacFaddin (1980). Confirmatory tests for gram negative bacteria were carried out using the API 20E system (API International, 65 Rue de la Prulay, 1217 Meyrin, Geneva, Switzerland).

Isolates were checked for histamine production using a differential medium containing histidine devised by Niveo, Jeffrey and Coriell (1981).

Pure cultures were inoculated into duplicate tubes of fish infusion broth (FIB), prepared from 100 g of fresh mackerel, according to the method of Arnold, Price and Brown (1980). FIB cultures

were incubated for 7 days at 37 and 20°C; and for 25 days at 7 and 1°C. Samples for histamine analysis were taken everyday for samples incubated at 37 and 20°C; and every 5 days for samples at 7 and 1°C.

2.3 Histamine Analysis

Histamine production in the FIB cultures was determined using a Turner Fluorometer III by the method described by Taylor, Lieber and Leatherwood (1978).

3. RESULTS AND DISCUSSION

The standard plate counts at 37°C of bullet mackerel flash held at ambient temperature for 24-48 h ranged from 9.8×10^6 - 8.1×10^5 /g. The composition of the microflora is shown in Table 1. Seventy percent (70%) of the bacteria isolated were *Proteus* spp. and 95% were histidine decarboxylase positive.

P. morganii incubated at 37 and 20°C rapidly produced high concentrations of histamine within 1-2 days, followed by a rapid decrease as shown in Figure 1. Maximum levels of 687 mg% occurred in cultures incubated at 20°C. Similar results were obtained by Arnold, Price and Brown (1980) for skipjack tuna. On incubation for 7 days at 37 and 20°C histamine levels fell to 276 mg% and 368 mg% respectively. This reduction is probably due to bacterial histaminase production, which has been reported to establish an equilibrium between histamine formation and destruction in foods containing large amounts of histamine (Ienistee, 1973). At 7°C the histamine concentration reached 65 mg% after 25 days and negligible amounts at 1°C for the same period (Figure 1).

Table 1

Identification of the 40 isolates from spoiled bullet mackerel

Species	%	Number	Histidine decarboxylase positive isolates
<i>Proteus</i> spp.	70	(28)	28
<i>Enterobacteriaceae</i>	2.5	(1)	1
<i>Pseudomonas</i> spp.	5	(2)	0
Gram positive rods	12.5	(5)	3
Unidentified	10	(4)	4

Histamine formation by *P. vulgaris* and *P. mirabilis* was very much lower as compared to *P. morganii* (Figures 2 and 3). The formation of histamine in skipjack tuna by *P. morganii* and *P. mirabilis* at 38°C has been reported to be 2.6 $\mu\text{g}/10^6$ cells and 0.062 $\mu\text{g}/10^6$ cells respectively (Yoshinaga and Frank, 1982). The maximum level of histamine formed by *P. mirabilis* was 10 mg% after 7 days at 37°C, and *P. vulgaris* 3.2 mg% after 1 day followed by a rapid reduction (Figures 2 and 3). No histamine was produced at 1°C in 24 days.

The bacteria responsible for histamine production in bullet mackerel are mesophiles, nearly all of which belong to the family *Enterobacteriaceae*. These microorganisms do not appear as part of the natural microflora of newly caught scombroid fish, but will contaminate fish which are handled unhygienically. If fish are kept at ambient tropical temperatures for extended periods after catching, which are favourable conditions for mesophilic growth, histamine accumulation will occur.

These results show that immediate icing of fish will inhibit histamine formation by *Proteus* spp. in bullet mackerel, however Okuzumi, Okude and Amano (1982) have reported the occurrence of psychrophiles as part of the normal microflora of marine fish which are histamine-forming. A recent study at the Department of Fish Processing Technology failed to detect the occurrence of these N-Group Bacteria (Domingo, 1984).

4. REFERENCES

- Arnold, S.H. and W.D. Brown, Histamine (?) toxicity from fish products. *Adv. Food Res.*, 24:113-54 1978
- Arnold, S.H., R.J. Price and W.D. Brown, Histamine formation by bacteria isolated from skipjack tuna 1980 (*Katsuwonus pelamis*). *Bull. Jap. Soc. Sci. Fish.*, 46:447-51

- Cowan, S.T., Cowan and Steel's manual for the identification of medical bacteria. Cambridge, 1974 Cambridge University Press, 2nd. ed.
- Domingo, T., Isolation of psychrophilic and halophilic histamine forming bacteria from frigate mackerel (*Aurula thazard*). M.S. Thesis, College of Fisheries, University of the Philippines, Quezon City
- Frank, H.A., D.H. Yoshinaga and W.K. Nip, Histamine formation and honeycombing during decomposition of skipjack tuna, (*Katsuwonus pelamis*) at elevated temperatures. Mar.Fish.Rev., 43:9-14
- Ienistea, C., Significance and detection of histamine in food. In The microbiological safety of food, edited by B.C. Hobbs and J.H.B. Christian. London, Academic Press, pp. 327-43
- Jensen, M.H. and E. Schultz, Utilization of iron agar in determining the freshness of wet fish. 1980 Dan.Vet.Tidsskr., 63:314-8
- MacFaddin, J.F., Biochemical tests for identification of medical bacteria. Baltimore, Williams and Wilkins, 527 p.
- Niven, C.F., M.B. Jeffery and D.A. Corisell, Differential plating medium for quantitative detection of histamine producing bacteria. Appl.Environ.Microbiol., 41:321-2
- Okuzumi, M., S. Okuda and M. Awano, Isolation of psychrophilic and halophilic histamine-forming bacteria from *Scomber japonicus*. Bull.J.Soc.Sci.Fish., 47:1591-8
- _____, Occurrences of psychrophilic and halophilic histamine-forming bacteria (N-Group Bacteria) on/in red meat fish. Bull.J.Soc.Sci.Fish., 48:799-804
- Taylor, S.L., Monograph on histamine poisoning. FAO Codex Alimentarius Commission Rome, FAO, 1983 CX/FH 83/11:75 p.
- Taylor, S.L., E.R. Lieber and M. Leatherwood, A simplified method for histamine analysis of foods. 1978 J.Food.Sci., 43:247-50
- Yoshinaga, D.H. and H.A. Frank, Histamine producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*) Appl.Environ.Microbiol., 44:447-52

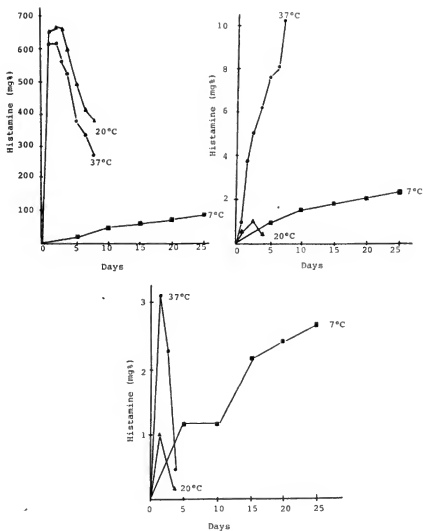


Figure Histamine production (mg%) by *P. vulgaris*, *P. morganii* and *P. mirabilis* at 37°C, 20°C and 7°C

RAPID SCREENING TEST FOR DETECTION OF *Proteus* spp.,
THE MAJOR HISTAMINE FORMERS IN FISH

by

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ABSTRACT

Since *Proteus* spp. are the most common histamine producing bacteria occurring in fish, the possibility of detecting the presence of large numbers of these species using a rapid urease reaction was examined. The results suggest that though *Proteus* strains varied in the rapidity of urease reaction, large numbers of cells gave a reaction in two-three hours. Fish homogenates inoculated with *Proteus* cells also gave positive urease reaction with the rapidity of reaction correlating well with the number of *Proteus* cells present. The effect of other bacteria likely to be associated with the fish, on the urease reaction by *Proteus* in broth was investigated. The results indicate that a positive urease reaction suggests the presence of a large number of *Proteus*, whereas negative reactions need further studies and that this test might be a useful screening test for fish likely to be involved in histamine poisoning.

1. INTRODUCTION

It is generally agreed that scombroid poisoning is caused by consumption of fish belonging to the families Scombridae and Scombaresocidae containing high levels of histamine. Fish can contain dangerous levels of histamine even when it is organoleptically acceptable (Arnold and Brown, 1978) and such fish are a public health hazard. The histamine problem is more acute in the fishmeal industry where the raw material is not preserved with as much care as fish for human use. Fishmeals containing histamine have been reported to cause stomach ulceration in chickens (Toyama et al., 1981) and in trout (Okuzumi et al., 1984). It has been well documented that histamine in scombroid fish is formed by bacteria that can decarboxylate histidine. Though several bacterial groups are capable of this activity, only a limited number of them has been actually isolated and identified from fishery products incriminated in scombroid poisoning (Eitenmiller, Joseph and Wallis, 1982). *Proteus morganii* has been most frequently mentioned in relation to histamine formation in fish muscle (Kawabata and Suzuki, 1959; Ferencik, 1970; Taylor et al., 1978) and currently, presence of high levels of *Proteus* is being used as a criterion of scombroid poisoning (Anon., 1972, 1975).

Proteus spp. are differentiated from other members of Enterobacteriaceae by their ability to rapidly hydrolyse urea. Though a number of other bacteria are also able to hydrolyse urea, the sensitivity of the test can be adjusted by the use of buffers (Blazevic and Ederer, 1975). Rustigian and Stuart (1941) devised a buffered medium in which a positive test specific for *Proteus* could be obtained in two-four hours. In this context, we investigated the possibility of using this medium for rapid screening of fish for the presence of large numbers of *Proteus* spp. The relation between the level of *Proteus* spp. in fish homogenates and rapidity of urease reaction, and the influence of other microflora that may be present in fish on this reaction were studied.

2. MATERIALS AND METHODS

Samples of mackerel (*Rastrelliger kanagurta*) were collected from the Mangalore fish-landing centre. They were analysed for total bacterial count by the surface spread method described by Speck (1976) and for histamine former count by the technique of Niven, Jeffrey and Corlett (1981). The generic distribution of histamine formers was studied by isolating and identifying 100 colonies appearing on the differential medium of Niven, Jeffrey and Corlett (1981), using the scheme of Lechavallier, Scider and Evans (1980).

Urease reaction of *Proteus* spp. isolated from fish were studied using the rapid urease test broth of Stuart, Van Stratum and Rustigian (1945), which consisted of 0.5% urea, 0.01% yeast extract and 0.004% phenol red in 1X phosphate buffer. The medium was sterilised by filtration through a membrane filter (0.45 µm, Millipore Corporation, USA). *Proteus* cells were grown in nutrient broth overnight, centrifuged and resuspended in saline and inoculated in urea broth to give inoculum

ranging from 10^2 to 10^8 cells/ml to study the relation between the number of cells in the inoculum and rapidity of urease reaction. After inoculation the broth was kept in a water bath at 37°C and change in colour noted every five minutes during the first two hours and hourly over the next 10 h and then overnight.

2.1 Urease Test as an Indicator of *Proteus* Counts in Fish Homogenates

To study the applicability of urease test directly to fish homogenates, a mackerel homogenate was prepared at 1:10 ratio with saline. Initial *Proteus* count in the homogenate was estimated using MacConkey agar. Ten non-lactose fermenting colonies were picked at random and subjected to urease and phenylalanine deaminase tests. Those positive for both were considered *Proteus* and based on the proportion of 10 colonies identified, the *Proteus* count in the homogenate was estimated. One millilitre homogenate was added to 5 ml urease broth to study the reaction at pre-inoculation stage. After this, 10 ml aliquots of a six-hour nutrient broth culture of *Proteus* was added to 100 ml homogenate, mixed well and incubated at 37°C for 24 h. Samples of contaminated homogenate for urease test were drawn immediately and after 12 h and 24 h. Urea broths were incubated at 37°C and the change in colour followed as mentioned above.

2.2 Effect of Other Bacteria on Urease Reaction of *Proteus* spp.

Since fish homogenates can be expected to contain large number of bacteria other than *Proteus*, the effect of these on the urease reaction of *Proteus* was studied. Two cultures of *Proteus* were used, one which gave a very quick urease reaction and the other was a slow reacting strain. Three millilitres of urea broth was inoculated with 10^6 cells of *Proteus* and 10^3 - 10^5 cells of following organisms, individually: *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Plesiomonas*, *Micrococcus*, *Bacillus* and *Staphylococcus* and the time taken for a positive urease reaction was determined.

3. RESULTS AND DISCUSSION

As shown in Figure 1, both strains of *Proteus* studied gave a positive urease reaction within three minutes when the inoculum was as high as 10^8 cells/millilitre. Strain A showed reaction within 160 min when the inoculum was 10^3 or more, while 10^4 or more cells of strain B were required to give a positive reaction in this time. However, even an inoculum of 10 cells of strain A gave a positive reaction in 10 h, while strain B required an inoculum of 10^3 cells to give reaction in 10 h. Both the strains were positive in 24 h. This suggests strain variation among *Proteus* spp. regarding the rapidity of urease reaction. Urease is considered a constitutive enzyme in bacteria (Burrows and Moulder, 1968) and is therefore produced by organisms even in the absence of the substrate. This may explain the immediate positive reaction when large numbers of cells were inoculated into broth. A small inoculum might not have sufficient enzyme and the late reaction is possibly due to multiplication of cells in broth and subsequent production of enzyme. The specificity of the urea broth of Stuart, Van Stratum and Rustigian (1945) is based on the premise that *Proteus* spp. are capable of multiplying by utilizing urea nitrogen and the essential growth factors from yeast extract, whereas other urease producing organisms require an additional nitrogen source (Cowan, 1974).

These results suggested that positive urease reactions occurred where fish homogenates containing a large number of *Proteus* cells were inoculated into urea broth, with the rapidity of reaction correlating with the number of cells present in the sample. This proposition is supported by results in Table 1, which shows that fish homogenates containing 10^3 cells/millilitre gave a positive reaction in two hours. When the *Proteus* level was raised to 10^7 cells/millilitre, a positive reaction was evident in 15 min. In fish samples with high concentration of histamine, *Proteus* levels of 10^6 to 10^7 /g have been reported (Okuzumi, et al., 1984). Results of the present study indicated that urease reaction could be directly tested on fish homogenates to give a quick indication of the presence of hazardous levels of *Proteus* spp.

Results in Table 2 suggested that various other bacteria present on fish might interfere with the urease reaction of *Proteus* spp. Different bacteria varied in their ability to delay the reaction by *Proteus* spp., but none of them completely suppressed the reaction of strain A. The reaction of strain B, on the other hand, was not only delayed by various bacteria, but even suppressed by *Bacillus*, *Micrococcus* and *Pseudomonas*. Though the urea broth of Stuart, Van Stratum and Rustigian (1945) has been reported to support multiplication of only *Proteus* spp. due to the lack of nitrogen sources other than urea, the specificity is partially lost when inoculated with fish homogenate, which contains ample nitrogen to support bacterial growth. This might explain the delay and suppression of reaction in some of the cases.

Nevertheless, the test might be a useful screening test for fish containing high counts of *Proteus* spp. While a positive reaction definitely indicates presence of a large number of *Proteus* spp., a negative reaction does not rule out the presence of a small number of *Proteus*, whose reaction might be suppressed by other bacteria; but since a large number of *Proteus* (10^6 - 10^7 /g) are usually present in fish containing significant levels of histamine, this test may be of use, as it is rapid, simple and does not require such expertise. The presently available methods of detecting histamine and histamine decarboxylating bacteria are time consuming and require expertise.

Our previous studies (Subburaj, Karunasagar and Karunasagar, 1984) have indicated a wide distribution of histidine decarboxylating bacteria in fish market environs in Mangalore. We examined the possibility of using rapid urease test to give an indication of *Proteus* counts in various samples from market environs, like water used for washing fish, ice, sweepings from market floor, fish-carrying baskets. The results (Table 3) indicated that except for a sample of ice, all other samples showed the presence of *Proteus* spp. and gave a positive urease reaction; whereas those negative for *Proteus* were also negative for urease reaction. This further strengthens the view that rapid urease test could be used as a quick screening test to indicate the presence of *Proteus* spp. in fish and fish market environs.

4. REFERENCES

- Arnold, S.M. and W.D. Brown, Histamine (?) toxicity from fish products. Adv.Food Res., 24:114-54 1978
- Blexevic, D.J. and G.M. Ederer, Principles of biochemical tests in diagnostic microbiology. 1975 New York, John Wiley and Sons
- Burrows, W. and J.W. Mouldar, Textbook of microbiology. Philadelphia, W.B. Saunders Company, 1968 Vol. I, 115 p. 19th ed.
- Cowan, S.T., Cowan and Steel's manual for the identification of medical bacteria. Cambridge, 1974 Cambridge University Press, 238 p. 2nd ed.
- Eitemiller, R.R., H.O. Joseph and W.W. Wallis, Histamine formation in fish, microbiological and biochemical conditions. In: Chemistry and biochemistry of marine food products, edited by R.R. Martin, et al. Westport, Connecticut, AVI Publishing Co., pp. 39-50
- Ferencik, M., Formation of histamine during bacterial decarboxylation of histidine in the flesh of some marine fishes. J.Hyg.Epidemiol.Microbiol.Immunol., 14:52-60
- Kawabata, T. and S. Suzuki, Studies on the food poisoning associated with putrefaction of marine products. VII. Distribution of L-(+)-histidine decarboxylase among *Proteus* organisms and the specificity of decarboxylation activity with washed cell suspensions. Bull.Jap.Soc. Sci.Fish., 25:473-80
- Lechavellier, M.W., R.J. Scider and T.M. Evans, Enumeration and characterisation of standard plate count bacteria in chlorinated and raw water supplies. Appl.Environ.Microbiol., 40:922-30
- Niven, C.F., Jr., M.B. Jeffrey and D.A. Corlett, Differential plating medium for quantitative detection of detection of histamine producing bacteria. Appl.Environ.Microbiol., 41:321-2
- Okuzumi, M., et al., Histamine forming bacteria in raw materials of fish meals. Bull.Jap.Soc.Sci. Fish., 50:883-8
- Rustigian, R. and C.A. Stuart, Decomposition of urea by *Proteus*. Proc.Soc.Exp.Biol.Med., 47:108-12 1941
- Speck, M.L., Compendium of methods for microbiological examination of foods. Washington, D.C., 1976 American Public Health Association
- Stuart, C.A., E. Van Stratum and R. Rustigian, Further studies on urease production by *Proteus* and related organisms. J.Bacteriol., 49:437-44
- Subburaj, M., I. Karunasagar and I. Karunasagar, Incidence of histidine decarboxylating bacteria in fish and market environs. Submitted for publication in Food Microbiology
- Taylor, S.L., et al., Histamine production by food-borne bacterial species. J.Food Saf., 1:173-81 1978
- Tayama, K., et al., Histamine content of fish meal. Bull.Jap.Soc.Sci.Fish., 47:415-9 1981
- Anon., Probable scombroid fish poisoning. Morbidity Mortality, 21:261-2 1972
- _____, Scombroid poisoning. New York City. Morbidity Mortality, 24:342-7 1975

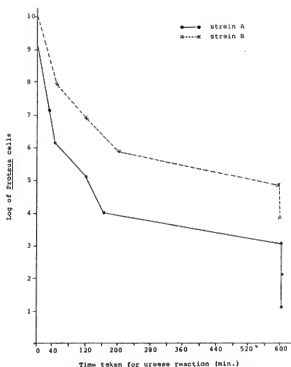


Figure 1 Concentration of calls vs. time for positive urease reaction

Table 1

Relation between the levels of *Proteus* in fish homogenates and the urease reaction in broth

	Pre-inoculation		Immediately after inoculation		12 h		24 h	
	<i>Proteus</i> count	Time taken (min)	<i>Proteus</i> count	Time taken (min)	<i>Proteus</i> count	Time taken (min)	<i>Proteus</i> count	Time taken (min)
Trial 1	0.76×10^3	120	0.30×10^7	15	0.1×10^8	15	0.45×10^9	10
Trial 2	0.88×10^3	120	0.51×10^7	15	0.8×10^8	15	0.2×10^9	10

Table 2

Effect of various bacteria on urease reaction of *Proteus* spp.

Organisms	No. of cells inoculated to urease broth	Time taken by <i>Proteus</i> (10 ⁶ cells) to give positive reaction	
		Strain A	Strain B
Nil		45 min.	120 min.
<i>Aeromonas</i>	1.1×10^5	4 h	10 h
<i>Bacillus</i>	6.9×10^4	4 h	-
<i>Flavobacterium</i>	1.3×10^5	4 h	10 h
<i>Micrococcus</i>	6.9×10^4	10 h	-
<i>Plesiomonas</i>	1.2×10^5	140 min.	10 h
<i>Pseudomonas</i> Strain 1	1.2×10^4	140 min.	140 min.
<i>Pseudomonas</i> Strain 2	2.1×10^3	140 min.	-
<i>Pseudomonas</i> Strain 3	3.8×10^4	140 min.	4 h
<i>Staphylococcus</i>	1.2×10^4	140 min.	140 min.

(-) No reaction in 24 h

Table 3

Proteus counts and urease reaction of various market samples

Sample		Histamine former count/ ml/sq. cm.	<i>Proteus</i> count/ ml/sq. cm.	Percentage incidence of <i>Proteus</i>	Time taken to give urease reaction
Water	1	5.4×10^6	5.4×10^5	10	10 h
	2	5.1×10^6	2.5×10^4	15	10 h
	3	5.4×10^6	5.4×10^5	10	10 h
Ice	1	1.2×10^5	1.2×10^4	10	No reaction
	2	7.0×10^5	Nil	-	No reaction
	3	4.1×10^5	Nil	-	No reaction
Basket	1	6.3×10^2	5.0×10^2	80	10 h
	2	5.5×10^2	4.1×10^2	75	10 h
	3	7.7×10^2	3.5×10^2	40	10 h
Floor	1	1.8×10^5	8.1×10^4	45	10 h
	2	2.3×10^5	1.3×10^5	55	120 min.
	3	2.4×10^5	1.9×10^5	80	10 h
	4	1.8×10^6	8.1×10^5	80	10 h

HISTAMINE FORMATION IN BOILED-SALTED (PINDANG) MACKEREL

by

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ABSTRACT

Boiled-salted fish (pindang) plays an increasingly important role in the Indonesian diet. However, short shelf life, slime, mould growth and quality improvements are the main handicaps to boost pindang production in Indonesia. In addition, histamine formation during handling and after processing are an unknown hazard especially for boiled-salted mackerel, although there were unofficial reports that some people have been suffering from histamine poisoning after consuming this product or tuna-like fishes. Studies on histamine formation in boiled-salted mackerel have been carried out. Effect of delayed icing on the quality and histamine formation were also studied and results are presented and discussed.

1. INTRODUCTION

Traditional production of boiled-salted fish (pindang) is one of the popular techniques of fish processing in Indonesia. Recently, about 5.81% (81 836 t) of the total fish catch, especially pelagic species, were processed into pindang. When compared to dried-salted fish, pindang is more popular and preferable, mainly due to its flavour and taste which resembles canned fish. In addition, the low salt concentration of pindang makes it a more acceptable product.

Traditional pindang processing involves boiling fish in saturated brine and marketing in non-hermetically sealed containers, which results in relatively short shelf life. It is suspected that serious public health problems may occur when poor quality raw material, particularly scombroid species such as mackerel, are used. High levels of histamine have been reported in tuna-like species and the problem of histamine toxicity is well documented (Taylor, 1983; Frank and Yoshinaga, 1982; Kimata, 1961). No data are available on the occurrence of histamine in pindang which may be important when considering quality and spoilage.

The purpose of this study was to investigate histamine formation in mackerel (*Rastrelliger neglectus*) during chilled and ambient temperature storage and to assess the quality of pindang prepared from raw material subjected to delayed icing.

2. MATERIALS AND METHODS

Fresh and uniform size of mackerel (*Rastrelliger neglectus*) with an average length, thickness and weight of 18.74 cm, 2.4 cm and 81.6 g, respectively, were used in this study. Pindang was prepared from raw materials which were subjected to delayed icing at ambient temperature (27.50-34.00°C, RH 62-85%) for 0, 3, 6 and 9 h and storage in crushed ice (0.70-2.30°C) for 0, 3, 6 and 9 days. Pindang processing was carried out by boiling the fish which were previously arranged in bamboo trays in boiling (104°C) brine (65% Salinometer) for 30 min. After the completion of boiling the pindang were drained and cooled to room temperature, followed by storage at ambient temperature (27.50-34.00°C, RH 62-85%). At certain time intervals samples were taken for measurements of pH, TVB (Convay Micro Diffusion Method), histamine analysis (Hardy and Smith, 1976), total plate count (TPC) and histamine producers count (O'Brien, Jeffrey and Corlett, 1981). Proximate analyses were carried out using the method of the AOAC (1980).

3. RESULTS AND DISCUSSION

3.1 Deterioration of Mackerel During Storage at Chill and Room Temperature

The raw materials contained 19.86% protein, 4.93% fat, 1.70 ash, 0.70% salt and 74.13% moisture.

Tables 1 and 2 show that histamine content of mackerel which were stored at room temperature (27.50-34.00°C) as well as chilled temperature storage (0.70-2.30°C) steadily increased with storage time and was accompanied by an increase in number of histamine-producing bacteria. It appeared

Table 1

Chemical and microbiological analysis of mackerel during storage at room temperature

Type of analysis	Storage time (hours)			
	0	3	6	9
pH	5.79	5.98	6.12	6.20
TVB (mg N%)	18.00	20.96	22.00	29.20
Histamine (mg %)	9.19	27.06	33.18	44.35
Total Plate Count (TPC)	3.0×10^4	7.3×10^5	8.0×10^6	1.4×10^8
Histamine Producers Count	2.2×10^5	3.5×10^5	1.1×10^6	3.5×10^6

Table 2

Chemical and microbiological analysis of mackerel during storage at chill temperature

Type of analysis	Storage time (days)			
	0	3	6	9
pH	5.79	6.27	6.20	6.33
TVB (mg N%)	18.00	21.71	18.80	22.31
Histamine (mg %)	9.19	9.62	17.82	23.08
Total Plate Count (TPC)	3.0×10^4	1.2×10^5	1.0×10^3	5.3×10^4
Histamine Producers Count	2.2×10^5	3.1×10^6	4.0×10^6	2.8×10^8

that the increase in histamine levels was due to the activity of microbial decarboxylation of histidine in the fish muscle as discussed by Frank, Yoshinaga and Pei Wu (1983).

In addition, the pH, TVB and TPC increase were accompanied by the increase of histamine-producing bacteria and an increase in histamine content. Evidently, the pattern of histamine formation is related to the extent of spoilage and directly associated with the activity of spoilage bacteria as suggested by Frank, Yoshinaga and Pei Wu (1983) and Taylor (1983).

The results of our study indicated that the rate of histamine formation under room temperature storage was much faster than under chilled temperature. This result supports previous findings discussed by Kimata (1961) which found that the optimum temperature for histamine formation was between 27.5°C and 34.0°C. The rate of histamine formation was inhibited by chilled temperature storage. Kimata (1961) and Sjaifullah (1978) also found that at 0°C histamine formation could be inhibited.

In our studies the chilled temperature ranged between 0.7°C and 2.3°C. Nevertheless, the histamine content of mackerel both kept at ambient and under chilled temperature storage was found to be 44.35 mg % (9 h at ambient temperature) and 23.08 mg % (9 days at chilled temperature) which are still below the toxic histamine level of 100 mg % suggested by Sjaifullah (1978).

As shown in Table 3, the effect of storage at ambient temperature on the formation of histamine was very significant. Fresh mackerel at 0 h had histamine content of 13.56 mg % and when subjected to storage for 3, 6 and 9 h, had histamine contents of 12.27 mg %, 17.21 mg % and 21.69 mg %, respectively. Although there were slight fluctuations there was evidence that the histamine content

Table 3

Chemical and microbiological analysis of boiled-salted mackerel made from raw materials which were stored at room temperature

Storage time of raw materials (hours)	Type of Analysis	Storage time of pickling at room temperature (days)			
		0	1	2	3
0	pH	6.36	6.25	6.34	6.49
	TVB (mg N%)	8.80	19.95	28.99	51.30
	Histamine (mg%)	13.56	17.40	15.70	17.94
	TPC	2.3×10^2	1.6×10^3	7.9×10^4	1.1×10^7
	Histamine Producers Count	0	0	0	2.0×10^2
3	pH	6.01	5.98	6.06	6.46
	TVB (mg N%)	14.13	30.73	32.18	36.40
	Histamine (mg%)	12.27	12.69	17.56	18.96
	TPC	9.2×10^3	4.0×10^2	2.3×10^6	2.1×10^6
	Histamine Producers Count	0	0	2.5×10^3	1.0×10^3
6	pH	6.02	6.08	6.00	6.36
	TVB (mg N%)	19.20	33.95	31.97	54.96
	Histamine (mg%)	17.21	16.38	28.50	19.25
	TPC	4.5×10^6	6.2×10^3	4.9×10^5	1.6×10^7
	Histamine Producers Count	0	1.1×10^2	5.5×10^4	2.8×10^3
9	pH	6.00	5.98	6.01	6.47
	TVB (mg N%)	26.39	34.91	31.89	67.76
	Histamine (mg%)	21.69	25.26	26.40	28.59
	TPC	4.7×10^3	5.4×10^5	2.0×10^5	4.2×10^6
	Histamine Producers Count	0	1.1×10^2	1.0×10^4	1.0×10^4

of pindang increased during storage. Since pindang processing involves cooking of fish in a boiling brine solution the increased level of histamine after processing was apparently due to recontamination, as also indicated by the increase of histamine-producing bacteria. The total volatile bases (TVB) and total plate count (TPC) also increased during storage. However, the TVB increase was not always accompanied by pH increase.

Pindang which were processed from raw materials stored at chilled temperature also gave a similar pattern of histamine formation. As shown in Table 4, histamine gradually formed after processing although at a slow rate. The TVB and TPC also steadily increased, but there were no significant changes in pH. On the other hand, the number of histamine-producing bacteria showed great variations. It was also recorded that some bacteria showed a negative test on Niven's agar under anaerobic conditions, but when reincubated aerobically they showed positive reaction. This phenomenon might be due to the inferior selectivity of Niven's agar or otherwise such species of bacteria only produce histidine decarboxylase under aerobic condition. The selectivity of Niven's agar is questionable since certain species of mould was able to grow and give a positive reaction on Niven's agar.

4. CONCLUSION

Chilling showed a significant effect on the histamine formation of fresh as well as boiled-salted (pindang) mackerel. Mackerel which were held under ambient temperature storage underwent rapid deterioration, accompanied by increased levels of histamine and histamine-producing bacteria. Chilling, in addition to decreasing the rate of fish spoilage, minimized histamine formation and inhibited the growth of histamine-producing bacteria.

After the cooking process followed by subsequent ambient temperature storage, the histamine content of pindang slowly increased obviously due to recontamination during storage.

The selectivity of Niven's agar is questionable since some species of mould was able to grow and show a positive reaction.

5. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1980 AOAC
- Frank, H.A. and D.H. Yoshinaga, Histamine producing bacteria in decomposing skipjack (*Katsuwonus pelamis*). J.Ser.Hawaii.Inst.Agric.Hum.Resour., (2641) 1982
- Frank, H.A., D.H. Yoshinaga and I. Pai Wu, Monograph for estimating histamine formation in skipjack 1983 tuna at elevated temperature. J.Ser.Hawaii.Inst.Agric.Hum.Reserv., (2696)
- Hardy, R. and J.G.M. Smith, Development of histamine and rancidity. J.Sci.Food Agric., 27:595-9 1976
- Kimata, M., The histamine problem. In: Fish as food, edited by G. Borgstrom. New York, Academic 1961 Press, vol. 1:329-52
- Niven, C.F., Jr., M.B. Jeffrey and D.A. Corlett, Jr., Differential plating medium for quantitative 1981 detection of histamine producing bacteria. Appl.Environ.Microbiol., 41(1):321-2
- Sjaifullah, I., Biokimia Ikan. Jakarta, Indonesia, Akademi Usaha Perikanan. 1978
- Taylor, S.L., Monograph on histamine poisoning. Codex Alimentarius Commission, Rome, FAO/WHO, 1983 CX/FH 83/11:75 p.

Table 4

Chemical and microbiological analysis of boiled-sealed suckler made from raw materials which were stored at chilled temperature

Storage time of raw materials (days)	Type of Analysis	Storage time of pindang at room temperature (days)				
		0	1	2	3	4
0	pH	6.36	6.25	6.34	6.49	6.15
	TVB (mg N%)	8.80	19.95	28.99	55.02	45.29
	Histamine (mg%)	13.58	17.40	15.70	17.94	15.75
	TPC	2.3×10^2	1.6×10^3	8.9×10^4	1.1×10^7	1.8×10^6
	Histamine Pro- ducers Count	0	0	0	2.0×10^2	6.0×10^2
3	pH	6.06	6.04	5.93	5.87	5.85
	TVB (mg N%)	17.09	22.80	23.44	25.19	23.47
	Histamine (mg%)	13.09	15.81	13.16	14.66	15.61
	TPC	9.2×10^3	9.7×10^4	1.0×10^4	1.0×10^7	3.5×10^6
	Histamine Pro- ducers Count	1.0×10^1	2.0×10^3	1.0×10^3	0	2.0×10^3
6	pH	6.08	6.05	6.44	5.99	5.80
	TVB (mg N%)	14.13	24.95	24.36	23.66	25.23
	Histamine (mg%)	15.69	19.77	20.78	19.94	24.67
	TPC	5.4×10^4	9.0×10^2	4.7×10^3	1.5×10^5	1.8×10^5
	Histamine Pro- ducers Count	0	1.1×10^3	5.5×10^3	0	0
9	pH	6.41	6.29	6.07	6.21	6.12
	TVB (mg N%)	19.85	22.90	23.90	24.08	26.13
	Histamine (mg%)	22.43	23.84	24.79	27.78	21.22
	TPC	4.7×10^3	1.0×10^2	4.1×10^4	1.6×10^6	2.0×10^6
	Histamine Pro- ducers Count	0	0	0	0	0

HISTAMINE FORMATION IN DRIED-SALTED MACKEREL

by

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ABSTRACT

The present study deals with histamine formation in dried-salted mackerel, a traditional fishery product. Effects of icing and salting on the quality changes and histamine formation were studied. Results on microbiological and chemical changes during storage were determined.

1. INTRODUCTION

Traditionally fish drying is an old and widely practiced method of fish preservation in Indonesia. About 33% of the total marine fish catch is converted into dried-salted fish (Table 1).

Table 1

Disposition and utilization of the marine fish catch

No.	Type	Disposition (t)	Percentage
1	Total catch	1 408 272	100
2	Fresh consumption	690 683	49
3	Salted/dried	464 944	33
4	Salted-boiled	81 836	5.81
5	Fermented products	61 073	4.33
6	Frozen	48 555	3.44
7	Smoked	34 901	2.47
8	Canned	10 561	0.73
9	Fish meal	4 856	0.34
10	Others	10 863	0.77

Dried-salted fish is popular in all regions but are mainly marketed in Java, particularly West Java. All kinds of fish can be processed into dried-salted products with various degrees of saltiness and with or without special preparation, such as splitting or dressing. The most common species which are dried and salted are trevallies, fringscale sardines, anchovies, rainbow sardines, oil sardines, mackerels, sharks, rays, scads, herrings, little tunas and squids.

Dried-salted fish is one of the nine main food items controlled by the Government since they play an important role in nutrition in the socio-economic status of most of the fishermen and fish processors, and the national price index of foodstuffs.

The scale of individual dried-salted fish production is usually small with traditional techniques, poor sanitation and hygiene. Consequently, the quality of the products is poor with a short shelf life. After several weeks of storage at ambient temperature dried-salted fish is subject to

mould growth, discolouration, development of off odours and pungent or bitter taste, especially for scombroid species.

It is suggested that histamine is formed in substantial amounts during the processing and storage period which might be responsible for the development of pungent or bitter taste after prolonged storage.

Since histamine content is often associated with the degree of freshness of fish and fishery products and has a direct impact on the consumers' health, studies on histamine content of dried-salted fish are important. The present study investigates the pattern of histamine formation during salting, drying and storage of dried-salted mackerel.

2. MATERIALS AND METHODS

2.1 Raw Material

The raw material used were fresh mackerel (*Rastrelliger negleotus*) caught by beach seine and iced on board. The fish was then transported to the Research Institute for Fishery Technology laboratory in insulated boxes with adequate icing.

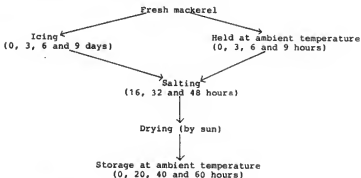
2.2 Salting-Drying

The fish were washed with clean water and divided into two lots. The first lot was iced with crushed ice (2:1) in insulated box, and another lot placed in plastic bag and held at ambient temperature. After storage, fish were salted four times, at intervals of 16 h with 30% salt (w/w).

The fish were placed in plastic buckets with alternating layers of salt and finally topped with salt and a weight, after which 10% of brine was poured into the salt-fish layers.

After drying, each batch of dried-salted fish were placed in plastic bags and stored at ambient temperature. At certain time intervals, samples were taken for quality assessment.

Schematically, the steps of the work are as follows:



2.3 Quality Assessment

Sensory evaluation (appearance, colour, odour, taste and texture) was carried out using a five-scale hedonic test.

2.3.1 Chemical analyses

- Histamine content was determined colourimetrically by coupling with a diazonium salt as suggested by Hardy and Smith (1976).
- Moisture, salt, protein, fat and ash contents were determined according to the methods of the AOAC (1980).
- Total volatile base (TVB) content and pH were determined according to AOAC (1980).

2.3.2 Microbiological analyses

- Histamine-producing bacteria were enumerated using Niven's method (Niven, 1981) and total plate count was determined using nutrient agar.

3. RESULTS AND DISCUSSION

The raw material used in this study was mackerel with an average length of 17.5 cm thickness and 75 g of weight. Using hedonic scale, the raw material had a score of 4.7 with moisture content of 75.2%, protein 19.7%, fat 4.2%, ash 1.4% and salt 0.99%. The initial pH of the raw materials was 6.2, with TVB and histamine contents of 18.0 mg/100 g and 1.46 mg/100 g, respectively. As far as microbiological quality was concerned, the total plate count of the raw materials was 3×10^4 and the histamine-producing bacteria was 2.2×10^5 .

3.1 Effects of Salting on Histamine

Mackerel subjected to salting for 16 h, 32 h and 48 h coupled with icing for 3 days, 6 days and 9 days, all show significant increase in histamine content (Figures 1-4). Surprisingly, the immediate formation of histamine during salting of fish iced for shorter periods was faster than that iced for longer periods. This is in contrast to the finding of Nasran *et al.* (these proceedings) who found that prolonged storage at room temperature accelerated histamine formation. It is suggested, therefore, that salt might play an important role in inhibiting the histamine formation or probably the growth or selection of histamine-producing bacteria. All batches subjected to prolonged storage at room temperature showed a slight increase in histamine formation during salting, which is in line with the finding of Nasran *et al.* (these proceedings).

3.2 Effect of Drying on Histamine Formation

After drying, all fish samples which were subjected to salting and icing or prolonged room temperature storage showed a slight increase in histamine content, indicating that histamine was still formed during salting and drying although in a slower rate.

3.3 Histamine Formation During Storage

During storage all samples of dried-salted mackerel showed insignificant increase of histamine levels and there was no evidence of histamine-producing bacterial growth (Figures 5-8).

It is apparent that the coupling effect of salting and drying suppresses the growth of histamine producers and histamine formation. The bactericidal effect of salt and reduction of moisture may inhibit the growth of histamine-producing bacteria.

4. CONCLUSION

Histamine might play a significant role as index of deterioration of dried-salted mackerel as well as incriminating agent of the development of pungent or bitter taste of dried-salted mackerel during prolonged storage.

Histamine was formed during the handling and salting process, but immediately ceased after drying. Unfavourable growth conditions after drying were responsible for the inhibition of the growth of histamine-producing bacteria and consequently inhibit histidine decarboxylation activities.

5. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1980 AOAC
- Hardy, R. and J.G.M. Smith, Development of histamine and rancidity. *J.Sci.Agric.*, 27:595-9 1976
- Niven, C.F., Jr., M.B. Jeffrey and D.A. Corlett, Jr., Differential plating medium for quantitative detection of histamine-producing bacteria. *Appl.Environ.Microbiol.*, 41:321-2 1981

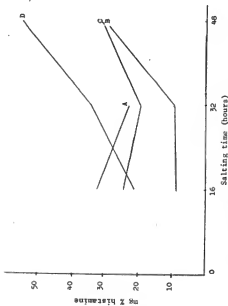


Figure 1 Histamine changes during salting

A - Raw material iced for 0 day
 B - " " " 3 days
 C - " " " 6 days
 D - " " " 9 days

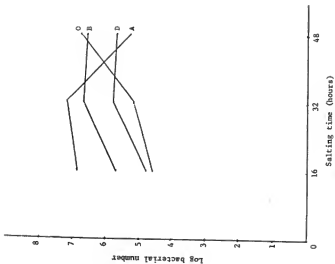


Figure 2 Histamine-producing bacteria counts during salting

A - Raw material iced for 0 day
 B - " " " 3 days
 C - " " " 6 days
 D - " " " 9 days

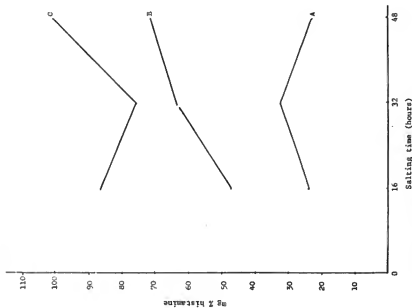


Figure 3 Histamine changes during salting
 A - Raw material kept in ambient temperature for 0 h
 B - " " " " " " 3 h
 C - " " " " " " 6 h

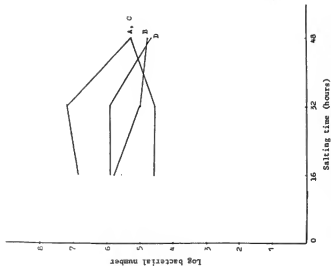


Figure 4 Histamine-producing bacteria count during salting

A - Raw material kept in ambient temperature for 0 h
 B - " " " " " " 3 h
 C - " " " " " " 6 h
 D - " " " " " " 9 h

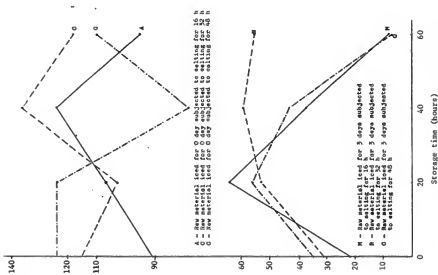


Figure 5a Histograms changes of dried-salted mackerel using iced raw material during storage

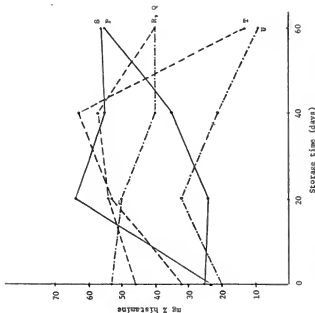


Figure 5b Histograms changes of dried-salted mackerel using iced raw material during storage

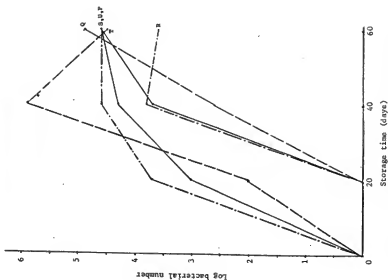


Figure 7a Histamine-producing bacteria of dried-salted mackerel using iced raw material during storage

A - Raw material iced for 0 day subjected to salting for 16 h
 C - " " " 0 day " " 32 h
 B - " " " 3 days " " 48 h
 D - " " " 16 h " " 16 h
 " " " 32 h " " 32 h
 " " " 48 h " " 48 h

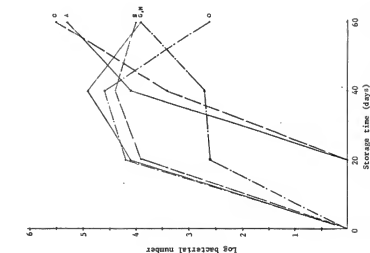


Figure 7b Histamine-producing bacteria changes of dried-salted mackerel using iced raw material during storage

P - Raw material iced for 6 days subjected to salting for 16 h
 R - " " " 6 days " " 32 h
 Q - " " " 6 days " " 48 h
 S - " " " 9 days " " 16 h
 T - " " " 9 days " " 32 h
 U - " " " 9 days " " 48 h

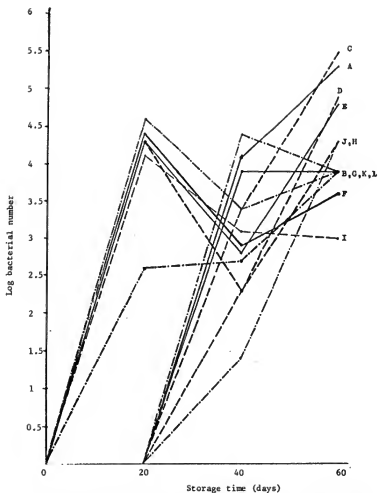


Figure 8 Histamine-producing bacteria changes of dried-salted mackerel using uniced raw material during storage

A	-	Raw material uniced for 0 h subjected to salting for 16 h
C	-	" " " " 0 h " " " " 32 h
G	-	" " " " 0 h " " " " 48 h
B	-	" " " " 3 h " " " " 16 h
D	-	" " " " 3 h " " " " 32 h
H	-	" " " " 3 h " " " " 48 h
E	-	" " " " 6 h " " " " 16 h
I	-	" " " " 6 h " " " " 32 h
K	-	" " " " 6 h " " " " 48 h
F	-	" " " " 9 h " " " " 16 h
J	-	" " " " 9 h " " " " 32 h
L	-	" " " " 9 h " " " " 48 h

STUDIES ON THE HISTAMINE CONTENTS OF FERMENTED FISHERY PRODUCTS

by

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ABSTRACT

Indonesian fermented fishery products were tested for the presence of histamine and results showed a wide variation, even within the same product type. Among the products studied, *peda* (fermented mackerel) had highest level of histamine ranging from 107.32 to 133.43 mg per 100 g. Some products had high volatile bases (TVB) and microbial contents. Nevertheless, sensory evaluation showed that all products tested were found acceptable to the trained panelists.

1. INTRODUCTION

In Indonesia, incidence of food poisoning due to the consumption of fish and fishery product is fairly high, although official data on the occurrence of outbreaks are scarce.

It is believed, although the casual relationships are not clearly understood, that one of the common substances responsible for food poisoning is histamine (Taylor, 1983; Kimeta, 1961). Histamine poisoning has historically been referred to as scombroid fish poisoning because of the frequent association of the illness with the consumption of spoiled scombroid fish. However, it has been reported that certain types of non-scombroid fish, such as mahi-mahi (*Coryphaena hippurus*), black marlin, sardines, anchovies and dorado, can also be involved in histamine poisoning. For this reason, the term scombroid fish poisoning is not entirely accurate despite many years of widespread use (Taylor, 1983). Other foods incriminated in incidents of histamine poisoning are fermented products (fermented fish, fermented-dry sausage, cheese, sauerkraut) and shellfish as well as chicken (Taylor, 1983; Taylor and Speckhard, 1983).

Histamine is formed in fish from histidine through an enzymatic decarboxylation reaction catalysed by histidine decarboxylase (Kimeta, 1961; Zaitsev, et al., 1969; Taylor, 1983). Early studies suggested that histamine was generated during autolysis (Kimeta, 1961). More recently, however, studies showed that the action of enzymes inherent in the muscle tissue of scombroid fish and certain non-scombroid fish only yields very little histamine (Geiger and Borgstrom, 1962). They found that the maximum amount of histamine produced during autolysis, even under optimum conditions with regard to pH and temperature, hardly exceeded 10 to 15 mg/g of meat. These amounts are negligible compared to those which may be produced by bacterial action (Kimeta, 1961). Therefore, since that amount of histamine in fish meat increases with the advance of spoilage, it can be concluded that the bacterial action plays greater role in histamine production (Kimeta, 1961; Zaitsev, et al., 1969).

Histamine-producing bacteria decarboxylate histidine in fish muscle under aerobic as well as anaerobic conditions. The enzyme histidine decarboxylase, is not widely distributed among bacteria and only found in certain *Enterobacteriaceae*, *Clostridia* and *Lactobacilli* (Taylor, 1983). Furthermore, enteric bacteria appear to be the most important histamine-producing bacteria in fish and *Proteus morganii*, *Klebsiella pneumoniae* and *Bifidobacterium* are the only bacteria that have been isolated from fish implicated in histamine poisoning incidents (Taylor, 1983; ICMSF, 1980).

Symptoms of histamine intoxication have been reviewed by Taylor (1983) and Goth (1976). Most countries do not have firm standards for levels of histamine in fishery products (Taylor, 1983). The United States has a 2-stage limit for histamine in canned tuna, a hazard limit of 50 mg% and a defect action limit of 20 mg%. In Indonesia, there is a lack of regulatory limits for histamine in seafoods. The subject of this paper was to study the level of histamine and histamine producing bacteria in fermented fishery products, using the standard of 50 mg% histamine for comparative purposes.

The fermented fish products studied were *jombal*, which is made from *Tachyurus* sp. and *peda*, a fermented mackerel (*Rastrelliger* sp.). Fish pastes included *petis* and a product made from shrimp *terasi*. A locally produced fish sauce and an imported sauce from Taiwan were also studied.

2. MATERIALS AND METHODS

Materials used in this study were fermented fishery products largely consumed in Indonesia. The samples were collected from several markets in Jakarta and its vicinity. All samples used had no onset signs of spoilage.

The histamine content of the samples was determined colourimetrically by coupling with a diazonium salt as suggested by Hardy and Smith (1976). Total volatile base (TVB) content was determined according to the method of the AOAC (1980). Histamine-producing bacteria of the samples were determined according to the method of Niven *et al.* (1981). Coliform and faecal coliform count was enumerated using the method described by Sumner (1981). Total plate count, *Staphylococcus*, *Listeria*, *Salmonella*, mold and yeast counts were also carried out. Organoleptic tests were carried out using a five-scale hedonic test by a trained taste panel.

3. RESULTS AND DISCUSSION

The histamine content of different fermented fishery products varied, even within the same kind of products (Table 1).

The results showed that among the products studied, only pada (fermented mackerel) has very high levels of histamine ranging from 107.32 to 133.43 mg/L. This is much higher than the proposed 50 mg/L comparison limit and may be potentially toxic. The high level of histamine in pada is mainly due to the high content of free histidine in raw material used. As a comparison, jambal (fermented *Thyrsurus* sp.), a similar product but non-scombrotoxic, only contains low level of histamine. A low level of histamine was found in fish pasta (petis and tarasi) and fish sauce which were locally produced and imported from Taiwan.

Salt apparently plays an important role in inhibiting the production of histamine as histidine is decarboxylated by members of the *Enterobacteriaceae* which are non-halophilic. No clear relationship was established between histamine and salt levels in this study (Table 1) and the numbers of histamine producing bacteria were generally low when compared to total plate counts (Table 2).

The total volatile bases are products of bacterial decomposition and levels were relatively high for the samples studied with the exception of petis and fish sauce from Taiwan. Lower bacterial numbers were found in both these samples when compared to the other products examined (Table 2). Highest counts were found on shrimp paste 7×10^5 to 3.6×10^6 /g. A TVB level of 130 mg/L or higher is undesirable in fermented fish products (Mackie, 1971), however levels reported here are generally higher and yet were acceptable to the taste panelists.

Pathogenic organisms such as *Salmonella* were absent from all products and numbers of *Staphylococcus* were within acceptable limits, with the exception of the fish sauce from Taiwan (Table 2). Faecal coliforms were also absent from all products. Other groups of organisms screened for were all within acceptable limits for these products (Table 2).

4. REFERENCES

- AOAC (Association of Official Analytical Chemists)., Official methods of analysis. Washington, D.C, 1980 Association of Official Analytical Chemists
- Geiger, E. and G. Borgstrom., Fish protein - nutritive aspects. In Fish as food, edited by 1962 G. Borgstrom. New York, Academic Press, vol. 2
- Goth, A., Medical pharmacology: principles and concepts. Tokyo, Toppan Co. Ltd., pp. 180-9 1976 8th ed.
- Hardy, R. and J.G.M. Smith., The storage of mackerel (*Scomber scombrus*) development of histamine and 1976 rancidity. *J. Sci. Food Agric.*, 27:595-9.
- ICMSF (International Commission on Microbiological Specifications for Foods), Microbial ecology of 1980 foods. vol. 2 Food commodities, edited by J.H. Silliker, *et al.* New York, Academic Press
- Kinats, M., The histamine problem. In Fish as food, edited by G. Borgstrom. New York, Academic 1961 Press, vol. 1: 329-47
- Mackie, I.M., R. Hardy, and G. Hobbs., Fermented fish products. *FAO Fish. Rep.*, (100): 54 p. Issued 1971 also in French and Spanish

- Niven, C.F. Jr., M.B. Jaffrey, and D.A. Corlett Jr., Differential plating medium for quantitative
1981 detection of histamine-producing bacteria. Appl. Environ. Microbiol., 41(1):321-2
- Sumner, J., Advanced microbiology: methods for detecting organisms of public health significance.
1981 Melbourne, Department of Food Science and Food Technology, RMIT
- Taylor, S.L., Monograph on histamine poisoning. Codex Alimentarius Commission. Rome,
1883 FAO/WHO, CX/FII 63/11: 75 p.
- Taylor, S.L. and M.W. Speckhard., Isolation of histamine-producing bacteria from frozen tuna.
1983 Mar. Fish. Rev., 45(4-5):35-9
- Zaitsev, V., et al., Fish curing and processing. (Transl. A. de Merindol). Moscow, MIR Publisher.
1969

Table 1

Histamine, TVB, salt and moisture content of fermented fishery products

Kinds of product	Histamine (mg %)	TVB (mg %)	(%) Salt	(%) Moisture
Fermented Fish				
- Jambal	11.27-27.74	153.40-257.19	12.61-13.29	48.06-49.53
- Peda	107.32-133.43	126.29-194.99	10.99-14.41	49.42-56.78
Fish Paste				
- Petis	16.36-28.44	48.08-50.75	2.58-8.01	20.37-28.45
- Terasi	1.20-24.22	316.72-325.88	5.86-17.34	33.04-44.08
Fish Sauce				
- ex Local	14.41-22.62	233.05-243.46	19.70-22.35	66.67-69.70
- ex Taiwan	4.93-10.74	18.01-10.74	21.16-23.67	66.60-73.54

Table 2

Microbiel content of fermented product

Product type	Total plate count ($\times 10^3$)	Histamine producers ($\times 10^3$)	Lactobacilli ($\times 10^7$)	Staphylococcus ($\times 10^7$)	Coliforms ($\times 10^3$)	Moulds ($\times 10$)	Yeasts ($\times 10^3$)
Fermented fish							
- Jambal	14-96	1.0-2.2	0.6-2.9	0.40-0.095	0-0.024	5.0	24.0
- Peda	3-10	2-9	0.08-0.20	0-2	0	5.0	23.0
Fish paste							
- Petis	0.7-1.4	0	0	0	0	36.0	0
- Terasi	700-3,600	0	0	0	0	0.8	0
Fish sauce							
- ex Local	41-700	1.7-5.0	0	0	0	20.0	0
- ex Taiwan	0.45-0.48	0-7.1	0.01-0.70	24-100	0.20-0.31	3.5-81.0	7.1

Note : Faecal coliforms and Salmonella were absent from all samples

HISTAMINE IN SOUTHEAST ASIAN CURED FISH AND CHANGES IN
HISTAMINE LEVELS DURING SALTING AND DRYING

by

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ABSTRACT

Histamine in cured fish samples from Southeast Asia was determined using the diazotised p-bromoniline colorimetric method. Over half the samples had histamine levels above 20 mg/100 g of fish flesh and one sample contained over 400 mg/100 g of fish flesh. The build-up of histamine during the curing process was investigated using mackerel (*Scomber scombrus*). Increases in histamine were observed during salting and drying for both whole and gutted fish, with greater increases in general for whole fish. Some preliminary results were obtained for changes in histamine levels during the storage of salted/dried fish.

1. INTRODUCTION

The relationship between high histamine levels in the flesh of scombroid and clupeid fish and the incidence of scombrototoxic poisoning has been reviewed by several authors (Arnold and Brown, 1978; Taylor, 1983). It is generally accepted that histamine is produced post mortem in fish flesh by bacterial decarboxylation of the amino acid histidine, which is found in high concentrations in the flesh of pelagic fish. Although compounds other than histamine appear to be necessary for scombrototoxic poisoning to occur, high histamine levels have often been found in samples of fish flesh implicated in scombrototoxic outbreaks, and it is therefore considered a useful indicator of potential hazard. Proposed regulatory limits for histamine in fish flesh have varied from as low as 10 mg/100 g up to 100 mg/100 g. Several agencies have proposed a limit of 20 mg/100 g, however, it is unlikely that any limit will obtain general acceptance until more is known about the mechanism of scombrototoxicity and more data are available on histamine levels in fish products. Only a few results have been reported for histamine levels in salted/dried fish and no information is available concerning the effect of salting, drying and subsequent storage on histamine production.

2. MATERIALS AND METHODS

2.1 Materials

All the cured fish products were obtained from processors, wholesalers and retailers in West Java, Indonesia, except for a sample of smoked tuna (S11) from a processor in Malaysia, and a sample of salted/dried *Sardinella* (S13) from a wholesaler in the Philippines.

Atlantic mackerel (*Scomber scombrus*) was obtained from the Grimby fish market.

2.2 Analytical methods

Histamine was determined by the diazotised p-bromoniline colorimetric method of Hardy and Smith (1976). Some loss of histamine occurs during the ion-exchange resin clean-up procedure for samples with high salt levels. This was corrected for by obtaining calibration curves for standard solutions of histamine containing salt. Determinations were carried out in triplicate.

Moisture was determined by drying samples to constant weight at $105 \pm 2^\circ\text{C}$. Protein was determined by the Kjeldahl procedure using a Kjeltec Auto 1030 Analyser; the data given in the tables are "crude protein" calculated using a protein factor of 6.25. Lipid was determined by a modified Bligh and Dyer procedure. Ash was determined at 500°C and NaCl in the ash samples by silver nitrate titration using potassium chromate as the indicator. Water activities were determined at $25 \pm 0.1^\circ\text{C}$ using a Novasine EEJ-3A water activity meter. Mean values of triplicate determinations are given in the tables, with the following approximate errors: Moisture ± 1 , protein ± 1 , lipid ± 0.5 , ash ± 1 , NaCl ± 1 , water activity ± 0.01 .

2.3 Mackerel Salting, Drying and Storage

Batches of fish were treated according to the scheme shown in Figures 1. Not all the stages were carried out for each batch.

2.4 Salting

Whole or gutted mackerel were mixed with salt (5 times the weight of the fish) in a plastic box and left for 48 h at 30 or 20°C. The pickle was retained in the box. The fish were then washed with tap water to remove excess salt.

2.5 Drying

The whole salted mackerel were split through the dorsal side and washed with tap water after removal of the viscera and gills. The gutted fish were split through the ventral side. The split fish were transferred to a drying cabinet and dried at either 48°C for 96 h or 30°C for 120 h.

2.6 Storage

The dried/salted fish were left at room temperature in a plastic tray at 20°C. Histamine was determined after 2, 3 and 5 weeks storage.

3. RESULTS AND DISCUSSION

In Table 1 histamine concentrations and other analytical data are given for 24 cured fish samples from Southeast Asia. The histamine levels varied from less than 10 to 420 mg/100 g of fish flesh. The 13 samples with histamine levels above 20 mg/100 g (a common regulatory limit) were all species of Scombridae, Clupeidae or Engraulidae (assuming the mixed batch S 23 contained species from these families). The tuna sample, S10, with the highest level of histamine, was known to have been stored for a long period of time and this was also thought to have occurred with the *Sardinella* sample, S13.

No correlations were found between moisture levels, NaCl levels or water activities and histamine levels. The lipid levels of the Spanish mackerel (*Scomberomorus* spp.) were, however, found to correlate directly with histamine levels ($p < 0.05$). This may be a spurious correlation, but could be due to a seasonal variation in both lipid and histidine or to a slower uptake of salt (and hence slower bacterial suppression) by the fish containing a higher percentage of lipid.

The high levels of histamine observed in some of these do not necessarily mean that the samples would present a health hazard since "potentiators" appear to be necessary for histamine poisoning to occur (Taylor, 1983). Such potentiators may not be present in sufficient quantities in this type of fish product. This aspect needs to be further investigated.

The high levels of histamine in the cured fish samples may have developed before processing, during processing or during subsequent storage. This was investigated for salted/dried fish by determining histamine levels before processing and after salting, drying and subsequent storage. The fish species used in the investigations was Atlantic mackerel (*Scomber scombrus*).

The effect of salting was studied either directly on the fish as received from the supplier, or after 24 or 48 h held at room temperature in order to increase histamine levels before processing, i.e., simulating poor handling. The effect of gutting the fish before processing was also studied. Two different temperatures, 20 and 30°C, were selected for salting, although the lower temperature is more typical of the temperature found in salting tanks in Southeast Asia and therefore this temperature was used for most of the experiments. The effect of drying was studied initially at 48°C but it was found that at this temperature partial cooking of the fish flesh occurred and subsequent drying experiments were carried out at 30°C. The final batch was stored for five weeks at 20°C. The histamine levels were determined at each stage of the processing. The results, which are in each case mean values for three fish, are given in Tables 2, 3, 4 and 5. The salt-free dry weight values are calculated on the basis of the fish flesh having 30% salt-free dry matter before salting, 40% after salting and 55% after drying.

On leaving the fish for a period of 24 h prior to salting it can be seen that on average an approximately five-fold increase in the histamine level occurs for a further 24 h. This is in agreement with previous studies which indicate that histamine increases rapidly in storage at these temperatures (Arnold and Brown, 1978).

When whole fish were salted at 30°C (Table 2), the histamine levels were found to increase on average by about twenty-fold (about fifteen-fold SFDM basis). Repeating the salting experiment with both whole and gutted fish at 20°C (Tables 3, 4 and 5) resulted in smaller increases in histamine levels. An approximately eight-fold increase on average (six-fold SFDM basis) was observed for the whole fish and a five-fold (four-fold SFDM basis) for the gutted fish.

Drying at both 30 and 48°C resulted in an increase in the amount of histamine (Tables 2 and 5). At both temperatures the histamine increased on average about three-fold (SFDW basis).

In a preliminary study on changes in histamine levels during the storage of salted-dried fish, some of the fish after drying were stored at 20°C and histamine levels were determined after 2, 3 and 5 weeks (Fig. 2). The histamine concentrations during storage generally reflected how the fish were treated before salting, i.e., higher for those held for 24 h before salting and higher for the fish salted whole; however, in each case an increase in histamine content was observed over the storage period.

The increases in histamine levels that were observed in this investigation are probably due in part to bacteria with histidine decarboxylase activity, particularly in localized areas of the fish flesh where salt penetration and drying were relatively slow, but may also have resulted from the activity of bacterial decarboxylase enzymes even when bacterial growth has been suppressed. The mechanism of production of histamine under these conditions needs to be investigated further so that methods to prevent or minimize histamine production may be developed.

4. CONCLUSIONS

(1) High histamine levels were found in samples of cured fish from Southeast Asia. More than half of the fish analysed contained more than 20 mg histamine/100 g.

(2) An increase in histamine levels was observed for both whole and gutted fish during salting and drying. When the fish were held at room temperature to simulate poor handling, the increase was even higher than in those salted directly as received. Gutting the fish prior to salting slowed down the rate of histamine production and this clearly is of importance in preventing high histamine levels in the end-product.

(3) An increase in histamine levels was observed during storage after drying.

5. REFERENCES

- Arnold, S.H. and W.D. Brown, Histamine (?) toxicity from fish products. Adv. Food Res., 24:113-54 1978
- Hardy, R. and J.G.M. Smith, The storage of mackerel (*Scomber scombrus*). Development of histamine and rancidity. J. Sci. Food Agric. 27:595-9 1976
- Taylor, S.L., Monograph on histamine poisoning. Codex Alimentarius Commission. Rome FAO/WHO, 1983 CX/PH 83/11:75 p.

Table 1

Histamine concentrations, proximate analysis data and water activities for cured fish from Southeast Asia

Sample Code	Type of fish ^{a/}	Percentage wet weight basis					Water Activity	Histamine mg/100 g
		Moisture	Protein	Lipid	Ash	NaCl		
S1	<u>Scomberomorus</u>	44	35	9.7	14.6	11.0	0.81	15
S2	<u>Scomberomorus</u>	48	32	6.7	15.6	12.0	0.81	20
S3	<u>Scomberomorus</u>	47	31	11.0	13.2	9.9	0.84	50
S4	<u>Scomberomorus</u>	43	32	11.7	16.0	12.3	0.77	65
S5	<u>Scomberomorus</u>	40	31	12.6	17.0	13.2	0.73	55
S6	<u>Scomberomorus</u>	38	36	10.7	17.1	12.9	0.73	65
S7	<u>Scomberomorus</u>	47	31	5.7	18.3	14.8	0.77	15
S8	<u>Scomberomorus</u>	40	36	11.7	14.5	9.0	0.79	30
S9	<u>Rastrelliger</u>	48	26	7.2	20.4	14.2	0.76	50
S10	Tuna	43	34	3.3	18.9	13.2	0.74	420
S11	Tuna	21	70	3.7	3.9	<0.1	0.85	10
S12	<u>Sardinella</u>	41	35	3.4	20.1	12.5	0.76	75
S13	<u>Sardinella</u>	15	52	9.7	28.0	18.8	0.61	220
S14	<u>Sardinella</u>	43	37	4.5	18.9	11.3	0.74	70
S15	<u>Sardinella</u>	43	27	3.5	21.3	16.8	0.73	80
S16	<u>Selar</u>	51	32	6.4	15.5	9.0	0.85	<10
S17	<u>Sciaenidae</u>	50	31	1.9	20.3	14.8	0.77	<10
S18	<u>Stolephorus</u>	37	38	3.7	23.4	16.9	0.73	30
S19	<u>Leiognathus</u>	45	22	7.4	26.0	17.0	0.74	<10
S20	<u>Leiognathus</u>	43	26	4.9	25.4	18.8	0.75	<10
S21	<u>Leiognathus</u>	45	23	5.5	25.8	17.4	0.74	<10
S22	<u>Arius</u>	49	35	1.7	15.6	13.9	0.78	<10
S23	Mixed small fish	36	40	4.9	23.1	17.6	0.71	100
S24	Mixed small fish	45	26	3.3	27.4	23.1	0.73	<10

^{a/} All samples are salted/dried except for S11 which was smoked. Identification of the fish (to the genus level) was not possible for S10, S11, S17 and the mixed fish samples S23 and S24

Table 2

Histamine concentrations in the flesh of whole mackerel (Batch 1)
on salting (48 h at 30°C) and drying (96 h at 48°C)

Time in hours stored at 30°C before salting	Wet weight (WW) or salt-free dry weight (SFDW) basis	Histamine concentration (mg/100 g) ^{a/}		
		Before salting	After salting	After drying ^{b/}
0	WW SFDW	1 (0.1) 3	16 (3.0) 40	34 (1.9) 60
24	WW SFDW	4 (1.3) 13	85 (5.0) 210	510 (44) 930

a/ Standard deviations are given in parentheses

b/ The fish were eviscerated and split before drying

Table 3

Histamine concentrations in the flesh of whole and gutted
mackerel (Batch 2) on salting (48 h at 20°C)

Whole (W) or Gutted (G)	Time in hours stored at 20°C before salting	Wet weight (WW) or salt-free dry weight (SFDW) basis	Histamine concentration (mg/100g) ^{a/}	
			Before salting	After salting
W	0	WW SFDW	2 (2.0) 7	28 (0.6) 70
W	24	WW SFDW	9 (2.3) 30	100 (28) 250
W	48	WW SFDW	95 (15) 320	220 (13) 550
G	0	WW SFDW	2 (2.0) 7	6 (4.0) 15
G	24	WW SFDW	12 (6.6) 40	39 (21) 100
G	48	WW SFDW	50 (23) 165	145 (23) 360

a/ Standard deviations are given in parentheses

Table 4

Histamine concentrations in the flesh of whole and gutted mackerel (Batch 3) on salting (48 h at 20°C)

Whole (W) or Gutted (G)	Time in hours stored at 20°C before salting	Wet Weight(WW) or salt-free dry weight(SFDW) basis	Histamine concentration (mg/100g) ^{a/}	
			Before salting	After salting
W	0	WW SFDW	1 (0.5) 3	3 (0.9) 8
W	24	WW SFDW	2 (0.8) 7	36 (12) 90
W	48	WW SFDW	13 (3.0) 43	175 (66) 440
G	0	WW SFDW	1 (0.5) 3	2 (0.7) 5
G	24	WW SFDW	2 (0.9) 7	29 (6.7) 75
G	48	WW SFDW	12 (0.8) 40	120 (8.2) 300

a/ Standard deviations are given in parentheses

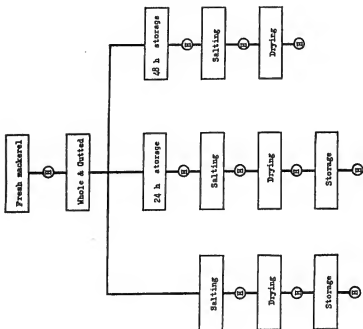
Table 5

Histamine concentrations in the flesh of whole and gutted mackerel (Batch 4) on salting (48 h at 20°C) and drying (120 h at 30°C)

Whole (W) or Gutted (G)	Time in hours stored at 20°C before salting	Wet Weight (WW) or salt-free dry weight(SFDW) basis	Histamine concentration (mg/100g) ^{a/}		
			Before salting	After salting	After drying ^{b/}
W	0	WW SFDW	2 (0.4) 7	5 (1.3) 13	20 (0.8) 36
W	24	WW SFDW	8 (0.2) 27	22 (1.0) 55	145 (19) 360
G	0	WW SFDW	2 (0.4) 7	3 (0.2) 8	6 (0.3) 15
G	24	WW SFDW	5 (0.1) 17	21 (0.2) 50	70 (0.5) 125

a/ Standard deviations are given in parentheses

b/ The fish were split before drying and the whole fish were eviscerated



(H) = Histamine analysis

Figure 1 Flow diagram for mackerel processing experiments

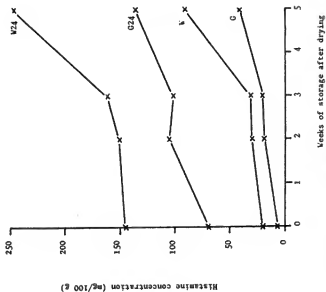


Figure 2 Changes in histamine levels during storage of salted/dried mackerel (Batch 4)

W24 - samples held 24 hours before salting whole

G24 - samples held 24 hours before salting gutted

W - samples salted directly whole

G - samples salted directly gutted

PRODUCTION OF FISH SAUCE FROM TWO SPECIES OF
INDIAN FISHES

by

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ABSTRACT

White bait (*Anchoviella* spp.) and carangid (*Caranx caranx*) were used for preparation of fish sauce, using the *nuoc-man* process of Cambodia. Both whole and minced fish were mixed with salt, in fish-to-salt ratio of 4:1, 3:1 and 2.5:1 and allowed to ferment over a period of 5-12 months. The supernatant liquid was skimmed off, filtered and bottled. The quality of the sauce was analysed after maturation over 5, 9 and 12 months under the sun and at room temperature. The chemical composition and quality of the final product after 12 months resembled *nam-pla* (fish sauce) of Thailand. The quality of sauce was better when the fish was allowed to ferment at room temperature. All the three fish-to-salt ratios yielded acceptable sauces, but that derived from 3:1 ratio was found to be the best. Both *Anchoviella* spp. and *Caranx caranx* yielded acceptable fish sauce.

1. INTRODUCTION

In Asia in general, and Southeast Asia in particular, a number of factors, particularly high temperature, favour the use of fermentation as a method of preservation of fish. It is inexpensive and in many instances whole fish are used. The demands on technology are slight and there is no requirement for chilling, storage or complex transport and distribution facilities. Fermented fish products like fish sauces, which are extensively used as a food source or as condiments in Southeast Asia, can become a means of utilization of increased fish landings in a simple and inexpensive manner. Converting fish into sauce can also be a better technique of preservation than drying in climates of high humidity. *Nuoc-man* of Viet Nam and Cambodia, *nam-pla* of Thailand and *patis* of the Philippines are some examples of typical fish sauces. In Thailand, the production of fish sauce in 1961 was 40 000 t from 33 000 t of fish (Saisithi, 1967).

The white bait (*Anchoviella* spp.), which are the most commonly used fish for production of fish sauce in Southeast Asia, are landed in large quantities by purse seiners along the Karnataka coast in India in recent years. During periods of glut, the price falls considerably, as most of the fish is used for drying since other preservation and utilization methods are unknown in India. The *Anchoviella* Study Team organized by FAO during 1982 has recommended the utilization of *anchoviella* as a raw material for fish sauce production for export to Southeast Asian countries. The results of preliminary investigations carried out in the College of Fisheries, Mangalore, in regard to the production of fish sauce from two species of Indian fishes, are reported in this paper.

2. MATERIALS AND METHODS

Fresh specimens of white bait (*Anchoviella* spp.) and a carangid (*Caranx caranx*) brought to the laboratory from the landing centre uniced, were thoroughly washed and divided into two batches. One batch was mixed whole with table quality salt with a fish-to-salt ratio of 4:1, 3:1 and 2.5:1 in duplicate glass jars with screw caps. The second batch of fish was minced and the mince was mixed with salt in the same ratios as in the case of the first batch and kept in duplicate sets of glass jars. Saturated brine (500 ml) was added after 0.5 h in order to cover the fish completely in brine and the bottle tightly closed. The fish and salt mixture in one set of jars was allowed to ferment at a temperature range of 38°-45°C under the sun and another set of jars was allowed to ferment at ambient temperature, 28°-33°C.

After a fermentation period of 5, 9 and 12 months, 200 ml of supernatant sauce was scooped off, filtered through coarse filter paper and analysed for quality parameters. Total nitrogen (TN) was estimated by microkjeldahl distillation (AOAC, 1975) and free amino nitrogen by the method of Pope and Stevens (1960). Total volatile base nitrogen was estimated by the method of Beatty and Gibbons (1937), while the salt content was estimated by the method suggested in FAO Technical Paper (FAO, 1981); pH was estimated by a double electrode pH meter and volatile acid value by AOAC (1975) method. The colour and odour were assessed by a taste panel.

3. RESULTS AND DISCUSSION

Fish sauce is a liquid of high salt content in which proteinaceous material of the fish has been degraded into free amino acids and nitrogenous bases such as ammonia, trimethylamine, urea and creatine. In all cases, fermentation takes place as a result of the action of proteolytic enzymes and microorganisms in the presence of high concentration of salt. The nature of the final product depends largely on the extent to which fermentation is allowed to proceed. In the present experiment the extent of fermentation, as indicated by total nitrogen, free amino nitrogen, total volatile bases, volatile acids and pH at the end of 5, 9 and 12 months is shown in Tables 1, 2 and 3. The salt content and description of sauce by sensory evaluation for odour and colour are presented in Tables 4 and 5. On the basis of these results, the following conclusions have been drawn up.

3.1 Effect of Fish to Salt Ratio on Rate of Fermentation

An examination of the data presented in Tables 1, 2 and 3 clearly reveals that the rate of fermentation was the fastest in samples with fish-to-salt ratio of 3:1 in all cases, irrespective of temperature of fermentation, duration of fermentation, species of fish and whether the sauce was from whole or minced fish. The data relating to total nitrogen content in sauce prepared from whole anchoviella at the three different fish to salt ratios and fermented under the sun over three different periods are shown in Figure 1, which clearly supports the above conclusion. Hamm and Clegue (1950) have recommended that *petis* should be made with sufficient salt to saturate the water content of fish and have recommended a fish-to-salt ratio of 3:1 in the present study. The high proportion of salt in the case of 2.5:1 ratio seems to have inhibited the rate of fermentation.

3.2 Effect of Temperature on Fermentation

Fermentation process, as any other chemical process, can be accelerated by raising the temperature to a certain extent during all or part of the fermentation period. Details of total nitrogen values of anchoviella sauce, prepared from whole fish and fermented under the sun and at room temperature with a fish-to-salt ratio of 3:1, are presented in Figure 2. This figure and the data given in Table 2 show that at this fish-to-salt ratio, the rate of fermentation was appreciably faster during early stages in samples exposed to sun than in samples fermented at ambient temperature in all cases. However, the differences narrowed down considerably with increasing duration of fermentation; but in the case of fish-to-salt ratio of 4:1, the trend was mostly just the reverse in all cases, while in samples with fish-to-salt ratio of 2.5:1, the trend differed with duration of fermentation, kind of fish and whether fermented from whole or minced fish. Up to 5 months, the rate of fermentation was fastest under the sun in all samples, while by the end of 9 months the extent of fermentation was greater for anchoviella samples at higher temperature and the reverse was true of carangid samples. At the end of 12 months, the extent of fermentation was more at room temperature in all samples, except in those of minced anchoviella.

The optimum temperature for fermentation by visceral enzymes is reported to be in the range of 34°-44°C (Freeman and Hoogland, 1956). The temperature range of 38°-45°C that is prevalent in coastal areas of South India seems to be favourable for fermentative processes, at fish-to-salt ratio of 3:1 only. The total volatile bases (TVB), however, were found to have appreciably increased in samples exposed to sun in all cases, while it was the reverse in the case of samples fermented at room temperature. Since increase in total volatile bases is due to breakdown of amino acids, it appears that fermentation at room temperature is preferable.

3.3 Whole Fish vs. Minced Fish

Grinding of fish with the visceral contents intimately mixes the meat with digestive enzymes and thus the rate of digestion and fermentation is likely to be accelerated. In the present experiment, the TN value of the majority of samples of sauce prepared from minced fish showed higher values, except in the case of samples with fish-to-salt ratio of 3:1 and fermented under the sun, where it was slightly lower.

3.4 Chemical and Nutritional Quality of Sauce

3.4.1 Total nitrogen and amino nitrogen

According to Subbarao (1967), first class puoc-nam should contain at least 16 g/litre of total nitrogen, out of which 50% should be formal nitrogen, and the ammoniacal nitrogen should not exceed 20-25% of total nitrogen. Total nitrogen content of nam-ple is reported to vary between 9.2 g/litre for low quality and 19.2 g/litre for high quality product, with corresponding values of 5.5 g/litre and 8.5 g/litre of amino nitrogen, respectively (Cluces, 1982). In the present experiment, the average TN values for sauces prepared from whole and minced fish fermented at room temperature were 12.23 g/litre in anchoviella sauce and 10.3 g/litre in carangid sauce. The corresponding average amino nitrogen values were 5.76 g/litre and 4.92 g/litre, respectively. On the basis of these values, these sauces resemble nam-ple. The TN and amino-N values of sauces fermented with fish-to-salt

ratios of 4:1 and 2.5:1 at room temperature and all the samples fermented under the sun were also found to be within the nam-pla range of values.

3.4.2 Total volatile bases

Ammonia and some amines are produced from amine oxides and degradation of amino acids present in fish. The ratio of these volatile bases to amino acid concentration can be used as an index of quality in fish sauce. A high ratio indicates bacterial spoilage and a poorer nutritional and organoleptic product. In the present experiment, amino nitrogen increased up to 9 months and thereafter it decreased with a concurrent increase in TVB values at the end of 12 months. TVB exceeded a value of 1 g/litre in samples exposed to the sun which, however, is within acceptable limits.

3.4.3 Volatile acids and pH

Low molecular weight fatty acids, like butyric and isovaleric, are said to be responsible for spoilage flavours in fish (Patton, 1964). However, in fermented products these acids are considered desirable. According to Saisichi (1967) and Tanikawa (1971), these acids are the main flavour imparting compounds in Thai sauce and sushi of Japan. Other low molecular weight acids, such as citric, ascorbic, succinic and lactic acids, when formed will confer characteristic flavours to the fish sauce. The anchoviella and carang sauces in the present experiment had average volatile acid values of 1.4 g/litre and 2.69 g/litre, respectively, when fermented with fish-to-salt ratio of 3:1 under room temperature. The volatile acid value of prime quality patis is reported to be about 5 g/litre (Nieto, 1982). The pH of both fish sauces varied between 6.0 and 6.5, which is a little higher compared to that of nam-pla, where it is reported to be less than 6.0.

3.4.4 Organoleptic quality of sauce

The salt content and the odour and colour description for fish sauces produced are given in Tables 4 and 5. The whole anchoviella sauce exposed to the sun lost its yellow colour only at the end of 12 months and became light brown, whereas ground anchoviella sauce changed to light brown at the end of 5 months.

Under room temperature, whole as well as ground anchoviella sauce changed to light brown colour only at the end of 5 months and gradually changed to brown under room temperature and dark brown under the sun. The brown colour of fish sauce is believed to be produced by non-enzymatic browning reactions (Seisithi, 1967). Brown colour to a certain extent is a desirable trait in fermented fish products.

Pleasant cheesy odour is said to be desirable in nuoc-nam (Mackie, Hardy and Hobbs, 1981). In the present experiment, the sauces from both species fermented under the sun gave out odour of self brine, characteristic of the fish, up to the end of nine months and thereafter changed to sharp pungent odour. The sauces fermented at room temperature developed pleasant cheesy odour at the end of nine months and further developed sharp but pleasant odour at the end of 12 months. From the biochemical and organoleptic analysis it appeared that even though fermentation process is completed at the end of nine months, a further maturation period is necessary for development of desirable colour and odour in the samples. On the whole, fermentation at room temperature appeared to yield a more acceptable product than when fermented under the sun.

On the basis of the foregoing discussion, it can be stated that the anchoviella and carangid resources of India can be successfully utilized for production of fish sauces acceptable to the Southeast Asian markets.

4. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official method of analysis. Washington, D.C., 1975 AOAC
- Beatty, S.A. and N.E. Gibbons, The measurement of spoilage in fish. J. Biol. Board Can., 3(1):77-91 1937
- Clucas, T.J., Fermented fish products - a review. In Fish handling, preservation and processing 1982 in the tropics. Part 2. London, Tropical Products Institute
- FAO, The prevention of losses in cured fish. FAO Fish. Tech. Pap., (219):87 p. Issued also in 1981 French
- Freeman, H.C. and P.L. Hoogland, Processing of cod and haddock viscera. Part 1. Laboratory 1956 experiments. J. Fish. Res. Board Can., 13(6):869
- Hamm, W.S. and J.A. Clague, Temperature and salt purity effects on the manufacture of fish paste 1950 and sauce. Res. Rep. U.S. Fish Wildl. Serv., (24):11 p.

- Mackie, I.M., R. Hardy and G. Hobbs, Fermented fish products. FAO Fish.Rep., (100):54 p. Issued 1971 also in French and Spanish
- Nieto, M.B., Factors affecting the ripening and flavour of bagoong alamang (shrimp paste) using 1982 *Acetiv* spp. MSc Thesis, University of the Philippines, Quezon City
- Patton, S., Flavour threshold of volatile fatty acids. J.Food Sci., 29:679 1964
- Pops, C.G. and M.F. Stevens, Determination of amino nitrogen using a copper method. Biochemistry, 1960 33:1070
- Saisithi, P., Studies on the origin and development of the tropical flavour and aroma of Thai fish 1967 sauce. Ph.D. Thesis, University of Washington
- Subbarao, G.N., Fish processing in Indo-Pacific area. Reg.Stud.FAO Reg.Off.Asia Far East, Bangkok, 1967 (4):231
- Tanikawa, E., Marine products of Japan: size, technology and research. Tokyo, Koseisha-Koseikaku 1971 Company, 305 p.

Table 1

Chemical parameters of fish sauce prepared with fish-to-salt ratio of 4:1 at the end of 5, 9 and 12 months

Fish used	Temp. (°C)	Period of maturation (months)														
		Total nitrogen (g/l)		Amino nitrogen (g/l)		Total volatile base nitrogen (g/l)		Volatile acids (g/l)		pH						
		5	9	12	5	9	12	5	9	12	5	9	12			
Anchoviella (whole)	25	9.80	9.27	10.12	4.67	5.86	5.23	0.68	0.69	0.84	0.13	0.21	1.73	6.5	6.0	5.9
Anchoviella (minced)	25	10.36	12.05	12.19	4.11	7.93	6.29	0.28	0.69	1.40	0.43	2.48	1.62	6.5	6.2	6.1
Catana (whole)	25	10.08	9.45	9.14	4.30	6.77	4.10	0.28	0.55	1.40	1.54	3.99	2.16	6.0	6.0	5.9
Catana (minced)	25	15.12	13.44	13.85	5.04	10.74	6.20	0.24	0.23	1.60	0.19	0.27	0.76	6.0	6.0	6.0
Anchoviella (whole)	25	11.90	13.40	13.89	5.60	9.29	6.02	0.84	0.32	0.39	0.92	1.41	2.38	6.5	6.3	6.0
Anchoviella (minced)	25	13.72	16.13	13.26	4.85	9.00	7.98	0.56	0.41	0.42	0.48	2.91	2.81	6.5	6.0	5.8
Catana (whole)	25	12.60	13.10	10.57	3.73	4.80	5.90	-	0.50	0.41	7.88	7.94	7.26	6.0	6.0	6.0
Catana (minced)	25	12.18	14.71	11.56	4.85	5.13	6.20	0.84	0.29	0.39	0.24	1.94	3.68	6.25	6.5	5.9

Table 2
Chemical parameters of fish sauce prepared with fish-to-salt ratio of 3:1 at the end of 5, 9 and 12 months

Fish used	Temp. (°C)	Period of maturation (months)															
		Total nitrogen (g/l)		Amino nitrogen (g/l)		Total volatile base nitrogen (g/l)		Volatile acids (g/l)		pH							
		5	9	12	5	9	12	5	9	12	5	9	12	5	9	12	
Anchoviella (whole)	25 28 25	14.56	12.99	12.72	5.04	8.14	6.02	0.28	0.88	1.40	0.12	2.65	2.05	6.5	6.0	6.1	
Anchoviella (minced)		14.28	11.65	11.92	3.55	7.42	5.69	0.84	0.70	1.40	1.97	0.43	2.16	6.5	6.2	6.1	
Caranx (whole)		18.48	15.23	12.63	5.23	8.21	5.93	0.88	0.82	1.40	0.97	5.51	5.65	6.0	6.5	6.4	
Caranx (minced)	28 25	17.92	14.29	13.80	5.23	11.85	5.55	0.84	0.21	1.50	1.03	0.43	1.08	6.0	6.0	6.1	
Anchoviella (whole)		10.64	10.62	11.47	4.48	6.35	5.23	0.40	0.21	0.21	0.27	0.32	0.87	6.5	6.3	6.1	
Anchoviella (minced)		12.18	14.61	12.99	4.48	8.26	6.30	0.28	0.26	0.21	1.10	2.20	1.96	6.5	6.0	5.8	
Caranx (whole)	28	10.08	10.70	8.42	2.99	5.74	4.29	0.84	0.43	0.36	5.40	5.40	3.99	6.0	6.2	6.0	
Caranx (minced)		14.42	14.84	12.19	4.48	8.73	5.55	0.40	0.39	0.21	3.88	4.64	1.40	6.0	6.5	6.2	

Table 3
Chemical parameters of fish sauce prepared with fish-to-salt ratio of 2.5:1 at the end of 5, 9 and 12 months

Fish used	Temp. (°C)	Period of maturation (months)														
		Total nitrogen (g/l)			Amino nitrogen (g/l)			Total volatile base nitrogen (g/l)			Volatile acids (g/l)			pH		
		5	9	12	5	9	12	5	9	12	5	9	12	5	9	12
Anchoviella (whole)	25	9.80	9.00	9.14	6.16	5.45	4.01	0.84	0.92	1.4	0.95	1.94	1.35	6.5	6.0	6.0
Anchoviella (minced)	45	12.60	15.59	15.32	5.04	10.92	7.97	0.28	0.70	1.6	0.30	0.64	1.08	6.5	6.2	6.0
Caranx (whole)	45	11.60	8.65	9.05	3.73	5.73	4.29	0.84	0.82	1.2	4.72	2.05	1.30	6.0	6.1	6.0
Caranx (minced)	35	15.40	11.55	11.74	4.67	9.79	6.57	0.28	0.13	1.15	0.45	0.32	0.43	6.0	6.0	6.0
Anchoviella (whole)	25	8.96	8.56	11.29	3.73	5.55	4.01	0.84	0.17	0.21	0.16	0.11	0.54	6.5	6.2	6.1
Anchoviella (minced)	45	10.98	13.10	11.46	3.55	5.58	5.69	0.28	0.43	0.21	0.43	0.18	0.97	6.5	6.0	5.8
Caranx (whole)	45	11.48	12.71	10.39	3.73	6.02	4.11	0.84	0.43	0.21	4.69	5.62	3.99	6.0	6.0	5.8
Caranx (minced)	25	12.04	14.17	12.19	3.73	9.89	6.58	0.84	0.22	0.24	0.86	1.29	1.08	6.25	6.0	6.2

Table 4

Salt percent and organoleptic characteristics of fish sauce fermented at 380-430°C (under the sun)

Type of fish	Fish to salt ratio	Average salt (%) in sauce	Organoleptic characteristics			
			Odour	Period of maturation (months)	Colour	
		5	5	12	5	12
<i>Anchoviella</i> (whole)	4:1	28.47	odour of self brine	odour of self brine	sharp, pungent	light brown
<i>Anchoviella</i> (minced)		28.25	odour of "	odour of "	pleasant, light cheesy, brown sharp	light brown
<i>Caranx</i> (whole)		28.68	odour of "	odour of "	sharp, pungent	brown
<i>Caranx</i> (minced)		27.15	odour of "	odour of "	pleasant, dark brown sharp	dark brown
<i>Anchoviella</i> (whole)	3:1	25.66	odour of "	odour of "	sharp, pungent	light brown
<i>Anchoviella</i> (minced)		28.36	odour of "	odour of "	sharp, pungent	light brown
<i>Caranx</i> (whole)		27.76	odour of "	odour of "	pleasant, yellowish sharp	brown
<i>Caranx</i> (minced)		27.60	odour of "	odour of "	sharp, pungent	dark brown
<i>Anchoviella</i> (whole)	2.5:1	27.60	odour of "	odour of "	sharp, pungent	light brown
<i>Anchoviella</i> (minced)		29.02	odour of "	odour of "	sharp, pungent	light brown
<i>Caranx</i> (whole)		28.60	odour of "	odour of "	sharp, pleasant	brown
<i>Caranx</i> (minced)		27.50	odour of "	odour of "	sharp, pungent	dark brown

Table 5

Percent of salt and organoleptic characteristics of fish sauce fermented at 28-33°C (room temperature)

Type of fish	Fish to salt ratio	Average salt (%) in sauce	Organoleptic characteristics			
			Odour	Period of maturation (months)	Colour	
			5	9	12	12
<u>Anchoviella</u> (whole)	4:1	22.50	odour of self brine	pleasant odour	pleasant, cheesy	yellow yellow yellowish brown
<u>Anchoviella</u> (minced)		26.87	"	pleasant "	pleasant, sharp	yellow yellow yellowish brown
<u>Caranx</u> (whole)		22.10	"	pleasant "	sharp, pungent	brown brown brown
<u>Caranx</u> (minced)		23.64	"	pleasant "	sharp, pungent	brown brown dark brown
<u>Anchoviella</u> (whole)	3:1	28.37	odour of self brine	pleasant odour	pleasant, sharp	yellow yellow yellowish brown
<u>Anchoviella</u> (minced)		28.50	"	pleasant "	pleasant, sharp	yellow yellow brown
<u>Caranx</u> (whole)		28.27	"	self brine"	pleasant, sharp	brown brown brown
<u>Caranx</u> (minced)		25.23	"	pleasant "	pleasant, sharp	brown brown brown
<u>Anchoviella</u> (whole)	2.5:1	28.98	odour of self brine	pleasant odour	pleasant, sharp	yellow yellow yellowish brown
<u>Anchoviella</u> (minced)		28.98	"	pleasant "	pleasant, sharp	yellow yellow brown
<u>Caranx</u> (whole)		28.28	"	self brine"	pleasant, sharp	brown brown brown
<u>Caranx</u> (minced)		28.90	"	pleasant "	pleasant, sharp	brown brown brown

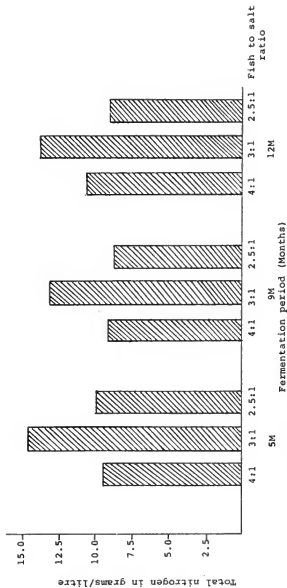


Figure 1 Rate of fermentation in relation to salting ratio during production of anchoviella sauce from whole fish under the sun (380-450C)

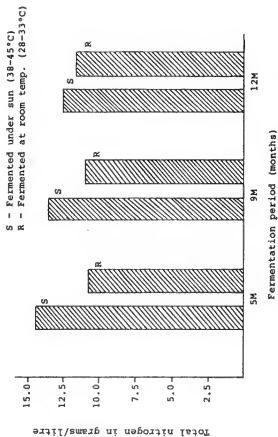


Figure 2 Rate of fermentation in relation to temperature and period of fermentation in anchovilla sauce prepared from whole fish with fish-to-salt ratio of 3:1

FISH SILAGE

by

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ABSTRACT

Thirty-three acid silages and 13 fermented silages were prepared from fish waste generated in the Melbourne area. Acid silages were of average composition 28.7% dry matter, 16.4% protein, 5.2% fat, 5.0% ash; fermented silages 33.6% dry matter, 14.4% protein, 6.4% fat and 6.7% ash. When utilized in the diet of pigs, acid and fermented silages were found to be highly digestible in terms of nitrogen and gross energy. Pigs fed acid silage in berley-based diets grew faster and more efficiently than control animals fed soyabean meal as a protein supplement. Silages were dried using wheat bran as a dehydration carrier. When included as sources of supplementary protein in the diets of chickens and salmon, acid and fermented silage meals produced growth and feed conversion not significantly different to control diets utilizing soyabean meal and fishmeal as sources of protein. Taste panel analysis indicated oily silage may be utilized in the diet of pigs and chickens without risk of taint, providing diets are carefully controlled.

1. INTRODUCTION

Fish silage is a brown, stable liquid stock feed prepared by acidifying whole fish or waste from fish processing. During manufacture fish are minced, acidified and stored until endogenous enzymes cause liquefaction of tissue. Acidification may be effected by direct acid addition to the mince (acid silage) or by generation of acid in the mince through fermentation of added carbohydrate (fermented silage). Although utilized as a major source of protein for animals in many countries, manufacture of fish silage in Australia is, at present, very much in its infancy with small amounts utilized as pig feed and as a foliar fertilizer.

In the present study, composition of acid and fermented silages prepared from filleting waste generated in the Melbourne area was determined and the nutritional value of silage, manufactured from local waste, for pigs, chickens and farmed fish assessed.

2. MATERIALS AND METHODS

2.1 Preparation of Silages

All silages were prepared from fish filleting waste obtained from the fish processors. Waste was refrigerated (4°C) or frozen (-40°C) until minced through a 2-mm plate and ensiled. Acid silages were prepared by addition of 3.5-litre formic acid per 100 kg of mince and fermented silage by addition of 12-15 kg of molasses and 5 litres of starter culture (*L. plantarum*) per 100 kg of mince. Batches ranged in size from 5 kg to 2 000 kg and silages were stored at room temperature (20°-25°C) or incubated at 30°C.

For incorporation in the diets of chickens and fish, silage was mixed with wheat bran (either 80-kg silage/20-kg bran or 85-kg silage/15-kg bran) and dried in an experimental batch dehydrator (6 h at temperature < 70°C). Dried silage meals were ground (2-mm plate) and stored in airtight containers prior to mixing with other feed ingredients. Acid silage meals (ASM) and fermented silage meals (FSM) were prepared.

2.2 Pig Digestibility Trials

Two feeding trials were conducted to determine digestibility by pigs of the dry matter, gross energy and nitrogen of acid and fermented silages prepared from mixed filleting waste. Coefficients of apparent digestibility were determined as the amount of nutrient ingested over a period of time by pigs, minus the amount voided in the faeces over that time expressed as a percentage of the nutrient ingested. Large white pigs of similar weight were housed in metabolism cages for a fixed

period of time (five-seven days) during which faeces were collected. Pigs were fed diets of barley in which silage was included at levels of 20% or 25% of the dry matter and on conclusion of feeding, digestibility coefficients were calculated. Digestibility coefficients were also determined for the barley meal and coefficients for silage determined by difference.

During the first trial the digestibility of nutrients in an acid silage, stored for six months prior to feeding, was determined. During the second trial digestibility of nutrients in acid and fermented silages, prepared from similar raw materials and stored (30°C) for two weeks prior to feeding, was determined.

2.3 Pig-Feeding Trial

A controlled pig-feeding trial was conducted during which 48 large white pigs were raised from 20 to 50 or 20 to 90 kg live weight. Four treatment diets were formulated; treatment 1 was a control diet utilizing soyabean meal as the entire source of supplementary protein. In the remaining diets soyabean meal was progressively substituted by acid fish silage, silage being the entire source of supplementary protein in the diet of treatment 4. Formulation and chemical composition of diets are presented in Table 1. Diets were designed to contain equivalent amounts of protein, lysine and digestible energy.

On attaining 50 kg live weight, six pigs/treatment were slaughtered and the meat produced subjected to taste panel analyses (triangle test and preference test) to determine the presence of taint. All remaining animals (six pigs/treatment) were slaughtered on reaching 90 kg although two stages of withdrawal of silage from diets were practiced in order to assess effect on flavour quality of meat produced. At 50 kg, three pigs/treatment were placed on the diet lacking silage (treatment 1) and at 70 kg remaining animals were placed on the diet lacking silage.

2.4 Chicking-Feeding Trial

Eight treatment diets were formulated to assess the nutritional value of acid silage meal (ASM) and fermented silage meal (FSM) in the diets of male broiler chickens. The formulation and chemical composition of the diets are presented in Tables 2 and 3. Treatment 1 was a control diet utilizing soyabean meal as the major source of supplementary protein, and treatment 2 was a further control diet in which the majority of the soyabean meal of the diet of treatment 1 was replaced by fishmeal (5%). Remaining treatment diets incorporated ASM or FSM substituting for soyabean meal to a maximum level of inclusion of 10%. Broilers were raised for 42 days on the treatment diets. On conclusion of feeding selected birds were slaughtered and meat produced subjected to taste panel analyses to assess any flavour alteration.

2.5 Fish-Feeding Trial

The nutritive value of acid silage meal (ASM) in the diet of chinook salmon (*Oncorhynchus tshawytscha*) was assessed. Four treatment diets were formulated in which fishmeal, the major source of supplementary protein in the control diet (treatment 1), was progressively substituted by ASM to a maximum level of inclusion of 30%. The formulation and chemical composition of the diets are presented in Table 4. Fish (initial weight 1.29 g) were held in 16 pens with 740 fish/treatment and were fed the diets for 52 days.

3. RESULTS

Analysis of the composition of a range of silages, prepared from fish filleting waste generated in the Melbourne area, indicated large variation in protein, fat, ash and dry matter contents, depending on source of offal. The average and ranges of values are presented in Table 5. In general, fermented silages were higher in dry matter and ash contents but lower in protein and fat contents than acid silages prepared from similar raw materials, this being an effect of substrate addition. Most silages contained high levels of oil (> 3%).

Coefficients of apparent digestibility (Table 6) for pigs, as determined during the digestibility trials, indicated acid and fermented silages were very digestible (> 80%) in terms of dry matter and gross energy, while the nitrogen in silages was highly digestible (96-97%). Little difference in digestibility of nutrients was found between an acid silage stored for six months (trial 1) or two weeks prior to feeding (trial 2), or between acid and fermented silages prepared from similar raw materials (trial 2).

Results showing the performance of animals during the pig-feeding trial are presented in Table 7. Statistical analysis indicated that during the 20-50 kg phase of growth, animals fed diets of treatments 2, 3 and 4 exhibited superior gain to those of animals fed the control diet (treatment 1). The superior performance by animals fed diets incorporating silage was reflected in the data for feed conversion of animals during this phase. There was no significant difference between treatments for animal performance during the 20-90 kg phase of growth.

Taste panel analysis indicated that of the pigs slaughtered at 50 kg, meat from animals fed the diet of treatment 4 was found to be significantly different in flavour to that of meat from animals fed the control diet (treatment 1) containing no silage and that the difference was detrimental. Meat from animals fed diets of treatments 2 and 3 and slaughtered at 50 kg, was not significantly different in flavour from that of control animals. Of the meat from animals slaughtered at 90 kg, that of pigs fed the diet of treatment 4, withdrawn at 70 kg, was found to be significantly different in flavour from that of control animals; however, withdrawal of the treatment 4 diet at 50 kg allowed sufficient time for reduction of off flavours in meat. Meat from pigs fed the diet of treatment 3 and slaughtered at 50 or 90 kg, was found to be not significantly different in flavour to that of control animals.

Results showing the performance of broilers during the chicken feeding trial are presented in Table 8. No significant difference was found between treatments for gain or feed conversion during any period of growth. Taste panel analyses conducted on meat from birds slaughtered after 42 days of feeding indicated that meat from animals fed diets of treatments 2 (5% fishmeal), 5 (10% ASM) and 8 (10% FSM) was not significantly different in flavour to that of animals fed the control diet lacking a protein supplement derived from fish.

In Table 9 are presented the results for performance of salmon during the fish-feeding trial. Although no significant difference was found between treatments for growth performance, a trend showing poorer gain and feed conversion by fish with increasing levels of inclusion of ASM was observed. Significant differences between treatments would be expected with prolonged feeding of the diets.

4. DISCUSSION

Variation in composition of silages, prepared from different fillsting wastes, indicates the necessity of routine analysis of silages by manufacturers/farmers to assure proper management of animal diets. Manufacture of silage from waste of a single species does not ensure a constant product as chemical composition, particularly fat content, has been found to vary seasonally (Gaiger, 1978).

The dry matter, gross energy and nitrogen of silages were very digestible to pigs. Digestibility of nitrogen was higher than that of commonly used protein supplements, which have apparent digestibility coefficients of nitrogen in the range of 78-90% (Leche, *et al.*, 1982).

Feeding trials indicated fish silage can be successfully incorporated in the diet of pigs, chickens and fish.

The significantly improved performance of pigs fed diets incorporating silage, compared with animals fed the diet utilizing soyabean meal as the entire source of supplementary protein, was most probably a reflection of greater availability of lysine in fish silage compared with soyabean meal. Chance and unidentified growth factors have also been postulated as causes when silage has produced superior growth in previous work (Batterham, Gorman and Chvojka, 1983). Feeding of a diet containing 2.5% fish oil (dry-matter basis, treatment 4) produced unacceptable flavour alteration in meat from animals slaughtered at 50 kg live weight, but such flavour alteration was not significant in meat from animals slaughtered at 90 kg if silage was withdrawn from the diet at 50 kg. Withdrawal at 70 kg was not sufficient to eliminate taint from meat of treatment-4 animals when slaughtered at 90 kg. Feeding the diet containing 1.6% fish oil (dry matter basis, treatment 3) resulted in the production of meat of flavour not significantly different to that of control animals when animals were slaughtered at 50 kg or when silage was withdrawn at 70 kg and animals slaughtered at 90 kg. This indicates that greater than 1% fish oil, regarded as the maximum level before taint is produced (Barlow and Pike, 1977), may be included in the diet of swine providing diets are fed for short periods or adequate withdrawal of silage from diets is practiced prior to slaughter.

ASM and FSM were successfully incorporated in the diet of broilers to a maximum inclusion of 10%. No toxicity of feeds incorporating silages was observed although the short periods of storage of silage and silage meals (10-14 days), and the use of fish waste of poor quality for silage production, may have prevented generation of harmful levels of toxic constituents previously found when feeding dried silage meals (Kompiang, Arifudin and Rao, 1980; Disney *et al.*, 1978). No evidence of leg weakness or vitamin deficiency in birds was observed.

The poor response of chinook salmon to ASM was disappointing although inferior performance of fish fed diets utilizing dried silage meals has been previously reported and attributed to low digestibility of meals (Hardy, Shearer and Spinelli, 1984). In the present study, poorer digestibility by fish of ASM compared with the fishmeal and the slightly lower protein content of the diets incorporating silage may have produced the tendency toward poorer performance of fish. Preparation of moist pellets by blending of wet silage with dry binder meal have been found to be very acceptable to fish producing good growth (Asgard and Austreng, 1981) and clearly warrants further investigation.

Table 1
Formulation and chemical composition (% dry-matter basis) of treatment diets
fed to large white pigs during silage feeding trial

	Treatment diets			
	1	2	3	4
Formulation				
Barley	82.7	83.6	84.5	85.5
Salt	0.27	0.27	0.28	0.28
Vitamin premix	0.27	0.27	0.28	0.28
Dicalcium phosphate	2.70	2.19	1.66	1.12
Soyabean meal	14.1	9.28	4.84	-
Acid silage	-	4.17	8.44	12.8
Chemical composition^{a/}				
Crude protein	17.4	17.5	17.6	17.7
Fat	2.1	2.9	3.6	4.4
Digestible energy (MJ/kg)	14.8	14.9	15.0	15.1
Calcium	0.92	1.01	1.10	1.18
Phosphorus	0.90	0.89	0.89	0.89
Lysine	0.95	0.96	0.97	0.98

a/ Diets of treatments 1, 2, 3 and 4 contained 88.9%, 80.9%, 74.0% and 68.2% dry matter, respectively

Table 2

Formulation and chemical composition (%) of starter diets fed to male broiler chickens between 1 and 21 days of age

	Treatment diets							
	1	2	3	4	5	6	7	8
Formulation								
Common ingredients ^{a/}	83.04	83.04	83.04	83.04	83.04	83.04	83.04	83.04
Soyabean meal	12.75	3.55	10.65	8.56	4.39	10.92	9.00	5.20
Fishmeal	-	5.00	-	-	-	-	-	-
ASM	-	-	2.50	5.00	10.00	-	-	-
FSM	-	-	-	-	-	2.50	5.00	10.00
Tallow	2.58	4.00	2.31	2.01	1.50	2.22	1.82	1.20
Dicalcium phosphate	0.95	0.42	0.71	0.48	-	0.70	0.59	0.13
Sodium chloride	0.26	0.21	0.23	0.21	0.15	0.20	0.18	0.08
Limestone	0.13	-	-	-	-	0.15	0.06	-
Methionine	0.25	0.18	0.25	0.23	0.21	0.24	0.25	0.23
Lysine	-	-	-	-	-	0.03	0.06	0.12
Rice hulls	0.04	3.60	0.31	0.47	0.71	-	-	-
Chemical composition								
Dry matter	87.5	87.8	88.9	88.1	88.4	88.6	88.2	88.5
Crude protein	21.1	20.9	21.9	21.0	21.3	21.7	20.9	21.3
Ether extract	7.3	8.2	7.7	6.8	7.7	7.5	7.5	7.9
Ash	4.9	5.1	4.8	4.5	4.5	4.7	4.5	4.5

^{a/} Common ingredients were as follows: wheat 53.0%, sorghum 10.0%, meat and bone meal 8.0%, whole sunflower seeds 5.0%, blood meal 2.54%, cottonseed meal 4.0%, vitamin premix 0.5%

Table 3

Formulation and chemical composition (%) of finisher diets fed to male broiler chickens between 22 and 42 days of age

	Treatment diets							
	1	2	3	4	5	6	7	8
<u>Formulation</u>								
Common ingredients ^{a/}	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Soyabean meal	12.75	4.64	10.65	8.56	4.39	10.92	9.00	5.47
Fishmeal	-	5.00	-	-	-	-	-	-
ASM	-	-	2.50	5.00	10.00	-	-	-
FSM	-	-	-	-	-	2.50	5.00	10.00
Tallow	5.11	6.13	4.84	4.54	4.03	4.75	4.42	3.76
Dicalcium phosphate	0.89	0.36	0.67	0.42	-	0.66	0.54	0.13
Sodium chloride	0.28	0.23	0.25	0.23	0.17	0.23	0.20	0.10
Limestone	0.24	0.20	0.20	0.16	-	0.20	0.08	-
Methionine	0.23	0.16	0.23	0.21	0.19	0.22	0.23	0.21
Lysine	-	-	-	-	-	0.05	0.07	0.14
Rice hull	0.50	3.28	0.66	0.88	1.22	0.47	0.46	0.19
<u>Chemical composition</u>								
Dry matter	86.3	88.8	88.1	88.4	88.2	88.5	88.1	88.7
Crude protein	19.1	19.6	19.7	19.0	19.3	19.7	19.5	19.3
Ether extract	7.1	7.6	7.3	7.9	7.7	7.5	7.5	0.8
Ash	4.6	4.8	4.8	5.1	4.7	4.3	4.6	4.1

^{a/} Common ingredients were as follows: wheat 50.5%, sorghum 15.0%, meat and bone meal 8.0%, whole sunflower seeds 3.0%, cottonseed meal 3.0%, vitamin premix 0.5%

Table 4
Formulation and chemical composition (%) of treatment diets
fed to chinook salmon (*Oncorhynchus tshawytscha*) during silage feeding trial

	Treatment diets			
	1	2	3	4
<u>Formulation</u>				
Common ingredients ^{a/}				
Fishmeal	42.1	42.1	42.1	42.1
ASM	40.0	35.9	32.0	27.9
Wheat bran	-	10.0	20.0	30.0
Rice hulls	12.8	8.5	4.3	-
	5.1	3.5	1.6	-
<u>Chemical composition</u>				
Dry matter	90.2	89.9	89.3	88.7
Crude protein	49.8	48.9	48.7	48.0
Gross energy (MJ/kg)	18.7	18.6	18.8	18.7
Calcium	2.34	2.33	2.47	2.49
Phosphorus	1.77	1.74	1.80	1.74

a/ Common ingredients were as follows: meat and bone meal 8.0%, soyabean meal 7.5%, blood meal 7.0%, skim milk powder 5.0%, cottonseed meal 5.0%, corn gluten 3.6%, salt 2.0%, linseed oil 2.0%, cod liver oil 1.0%, vitamin premix 1.0%

Table 5

Composition (% wet-weight basis) of acid and fermented silage^{a/}

	Acid silage		Fermented silage	
	Mean	Range	Mean	Range
Dry matter	29.2	18.8-38.7	33.6	29.6-39.1
Crude protein	16.4	11.6-23.7	14.4	11.9-16.8
Fat	5.0	0.6-15.6	6.4	1.9-14.2
Ash	5.3	1.9- 9.8	6.7	4.6-12.8

^{a/} 35 acid silages and 13 fermented silages were prepared

Table 6

Coefficients of apparent digestibility (%) determined for silages during pig digestibility trials

Trial	Type of silage	Coefficient of digestibility (%)		
		Dry matter	Gross energy	Nitrogen
1	acid	80	87	96
2	acid	82	88	97
	fermented	84	88	97

Table 7

Performance of large white pigs during silage feeding trial

	Treatment diets			
	1	2	3	4
(a) 20-50 kg phase of growth				
Liveweight gain (g/bird)	627a	641b	648b	651b
FCR (g feed/g gain)	2.13a	2.05b	2.00bc	1.98c
(b) 20-90 kg phase of growth				
(i) silage withdrawn at 50 kg				
Liveweight gain (g/bird)	772	789	802	775
FCR (g feed/g gain)	2.33	2.29	2.24	2.34
(ii) silage withdrawn at 70 kg				
Liveweight gain (g/bird)	798	813	830	815
FCR (g feed/g gain)	2.28	2.20	2.15	2.19

Within a parameter, differing subscripts indicate significant difference between treatments

Table 8

Performance of male broiler chickens raised during silage feeding trials^{a/}

	Treatment diets							
	1	2	3	4	5	6	7	8
(a) 0-21 days period of growth								
Liveweight gain (g/day)	674	642	653	652	676	657	661	650
FCR (g feed/g gain)	1.65	1.65	1.77	1.70	1.69	1.64	1.64	1.66
(b) 22-42 days period of growth								
Liveweight gain (g/day)	1186	1053	1187	1115	1146	1176	1136	1204
FCR (g feed/g gain)	3.07	2.97	2.98	2.96	3.05	2.99	2.84	2.82
(c) 0-42 days period of growth								
Liveweight gain (g/day)	1859	1694	1841	1763	1822	1833	1796	1854
FCR (g feed/g gain)	2.51	2.41	2.55	2.45	2.52	2.46	2.37	2.36

^{a/} No significant difference between treatments for gain or feed conversion ratios

Table 9

Performance of chinook salmon during silage feeding trials^{a/}

	Treatment diets			
	1	2	3	4
Liveweight gain (g/100 fish)	180	174	163	159
FCR (g feed/g gain)	1.35	1.41	1.50	1.52

^{a/} No significant difference between treatments for gain or feed conversion ratios

5. REFERENCES

- Asgard, T. and E. Austreng. Fish silage for salmonids: a cheap way of utilising waste as feed.
1981 Feedstuffs, 22
- Barlow, S.M. and I.H. Pike. The role of fat in fish meal in pig and poultry nutrition. Tech.Bull.
1977 Int.Assoc.Fish Meal Manuf.,U.K., (4):38
- Batterham, E.S., T.B.S. Gorman and R. Chvojka. Nutritional value and mercury content of fish silage
1983 for growing pigs. Anim.Feed.Sci.Technol., 9:169
- Disney, J.G., et al., Development of a fish silage/carbohydrate animal feed for use in the tropics.
1978 Trop.Sci., 20(2):129
- Gaiger, P.J., Fish silage trials in Hong Kong. Proc.IFFC, 18(3):527-42
1978
- Hardy, R.W., K.D. Shearer and J. Spinelli. The nutritional properties of co-dried fish silage in
1984 rainbow trout (*Salmo gairdneri*) dry diets. Aquaculture, 38:35-44
- Kompiang, I.P., R. Arifudin and J. Raa. Nutritional value of ensilaged by-catch from Indonesian
1980 shrimp trawlers. In Advances of fish science and technology, edited by J.J. Connell.
Farnham, Surrey, Fishing News (Books) Ltd. pp. 349-52
- Leche, T.F., et al., Composition of animal feedstuffs in Australia. Blacktown, N.S.W., Australia,
1982 Australian Feeds Information Centre, Division of Animal Production, CSIRO

CONSUMER ACCEPTABILITY OF SHARK

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ABSTRACT

Consumer acceptance of 12 species of shark from Northern Territory waters was assessed by comparison with well accepted cold-water species. *Mustelus antarcticus* (gummy shark) and *Galeorhinus australis* (school shark).

Evaluation was carried out by (1) sensory evaluation through taste panel tests and (2) objective evaluation through examination of chemical properties (hypoxanthine content, pH and moisture content) and physical properties (colour and texture).

Taste panels were able (70% of tastings) to distinguish between warm and cold-water species but the difference was rated "slight-moderate" and no preference emerged for either warm or cold-water shark. That is, about 50% of preferences were given to each species group.

Cold-water species were significantly whiter, smoother and moister in texture and had a blander flavour compared with the warm-water species which emerged as yellow/grey, having a firmer meaty texture and stronger flavour. These differences in colour, texture and moisture were confirmed in objective evaluation tests.

Only one warm-water species, *Carcharhinus amatus* (mangrove shark) was found to have poor consumer acceptability because when cooked it had a dry, rubbery texture and the flesh was darkly coloured.

The indications from this study are that there are intrinsic differences, warm-water species presented as more meaty, tangy compared with the whiter, softer, blander cold-water shark but both groups were equally acceptable to consumers.

1. INTRODUCTION

Of the approximately 17 000 t of whole shark presently harvested from Australian waters only some 10 000 t are landed in Australia for domestic consumption; major species are *Mustelus antarcticus* (gummy shark) and *Galeorhinus australis* (school shark), typically taken from the colder, southern waters and approximately 90% of which ultimately enter the Victorian market.

Estimates prepared by the Department of Primary Industry, Fisheries Division, Canberra, CSIRO, and the Department of Primary Production, Fisheries Division, Northern Territory, indicate a tropical shark resource of approximately 10 000 t, comprising mainly *Carcharhinus* spp. (black-finned school sharks), *Phizopronodon* spp. (milk sharks) and *Sphyrna* spp. (hammerheads) which could be harvested annually from the Timor and Arafura Seas for consumption both within Australia and overseas. This resource is presently being exploited (7 000 t annually) under foreign fishing arrangements (bilateral and joint venture) for marketing overseas.

The question has been asked - would the warm-water species of shark prove acceptable to the Victorian consumer? For several reasons the prognosis has been considered unfavourable. Firstly, for reasons of distance, shark landed in the Northern Territory would require freezing, frozen storage and refrigerated transport to Melbourne. Secondly, the entry of shark into Victoria would, of itself, necessitate, in the form of shark trunks - a marketing disadvantage given the Victorian preference for frozen shark as fillets, rather than in the round. And thirdly, a perception by Victorian buyers that consumers would consider warm-water shark as inferior to the colder southern species, in turn affecting demand and prices.

To test the preferences of Melbourne consumers for warm- and cold-water sharks, a series of taste panel evaluations was carried out by the Food Technology Unit, Royal Melbourne Institute of Technology, during 1983. As well, the project examined the physical and chemical characteristics of sharks to assess any intrinsic differences which might affect eating quality.

The project was funded under a grant from the Northern Territory Fishing Industry Research and Development Trust Fund.

2. MATERIALS AND METHODS

2. Raw Materials

Filets (unfrozen) of gummy and school shark of excellent quality were selected from stock offered for sale from Canals, Seafood Appreciation Centre, Melbourne. Unfrozen filets which were usually required the same day for consumer evaluation, were stored in ice. Filets were also frozen on trays in a blast freezer at -40°C , glazed in iced water and refrozen for 15 min before being vacuum packed and stored in the freezer until required.

Trunks of *Carcharhinus*, *Rhinochimaera* and *Sphyrna* (Table 1) caught in the Timor and Arafura Seas, were received still frozen by road transport from Darwin. They were thawed in a refrigerator overnight, skinned, filleted, refrozen and vacuum packed as above. All trunks were in good condition, with no evidence of freezer burn.

2.2 Taste Panel Evaluation

2.2.1 Triangle difference tests

Shark filets, wrapped in aluminium foil to ensure retention of juices, were baked without condiments at $250^{\circ}\text{C}/20$ min. Each consumer was presented with three samples of cooked shark, each sample labelled with a 3-digit code. Of the three samples, two were identical and the third from another species. The panelist was requested firstly, to identify the single sample, then to state a preference, and finally, to indicate the degree of difference between the single and the paired samples on an arbitrary scale from 0 to 12 (0 = no difference; 3 = slight; 6 = moderate; 9 = large; 12 = extreme difference).

2.2.2 Paired preference test

Experienced panelists were used to carry out preference tests between gummy shark and *C. amblyrhynchoides* (grey whaler shark) and school shark, and *S. blochii* (hammerhead). The aim of this test was to highlight any differences in colour, flavour and texture between the pairs.

3. RESULTS

Triangle testing indicated that taste panelists were able to distinguish between cold-water sharks (gummy and school shark) and warm-water sharks (range of species, see Table 1) whether the latter were compared with frozen or chilled cold-water shark (Table 2). Overall, of 1 040 evaluations, 723 (70%) correctly identified the "odd man out" of the three samples offered.

Of those able to distinguish the samples offered, 398/723 (55%) preferred warm-water shark and 325/723 (45%) preferred cold-water shark. These results demonstrate that there was no significant difference ($p < 0.05$) in consumer preference for either the cold- or warm-water shark.

Paired preference tests highlighted the dominant characters which allowed panelists to correctly distinguish cold- and warm-water sharks (Table 3). School and gummy sharks were significantly whiter, smoother and moister in texture and had a blander flavour compared with *C. amblyrhynchoides* and *S. blochii* which emerged as yellow/grey, having firmer, meaty texture (chewy/flaky) and stronger flavour (salty/seawaddy).

A number of laboratory tests confirmed the findings of the taste panelists. The Hunterlab Colour Difference Instrument established the white character of gummy and school shark. The moist, smooth, soft texture of the cold-water species was reflected in their higher moisture content both in the raw (75-77%) and cooked (72-73%) form, compared with warm-water species (73-76%) raw and (66-71%) cooked. The chewy, flaky characteristics of warm-water species was confirmed by the Instron Food Testing Instrument on which a shearing force of 34 kg was needed for *C. acronotus* compared with 15 kg for gummy shark.

Thus the preconception that Melbourne consumers would consider warm-water shark inferior was not borne-out.

Certainly taste panelists were able (70% of testings) to distinguish between warm- and cold-water species, but the difference was rated "slight-moderate" and no preference emerged for either

warm or cold-water shark. The indication from this study is that while there are intrinsic differences, warm-water species presenting as more meaty, tangy compared with the whiter, softer, blander cold-water shark, both types should be acceptable to consumers.

A major determinant of consumer acceptability appears to be handling, rather than a species difference. In one consumer trial, carried out in Darwin, warm-water species were judged significantly less acceptable than gummy shark. Of 115 panelists, 82 correctly identified the odd sample, 64/82 (78%) preferring gummy shark. The warm-water shark trunks used for this tasting had been stored, unglazed and unwrapped in a freezer store for six months and showed freezer burn. The cooked fillets were dark-coloured with a dry, rubbery texture which were compared adversely with the white, moist fillets of gummy shark.

Of importance in developing this fishery for acceptance within Australia is, firstly, the defining of appropriate standards for fishermen which will allow the discarding of fish which are likely to be downgraded on receipt at market because of defects like greening of gut cavity and odour formation (not ammonia). Secondly, the on-board and on-shore handling which will allow presentation of shark trunks acceptable to markets in Victoria and New South Wales requires definition.

Table 1

Shark species tasted for organoleptic quality

Scientific name	Common name	Marketing name
Cold-water species		
<u>Mustelus antarcticus</u>	Gummy Shark	
<u>Galeorhinus australis</u>	School Shark	
Warm-water species		
<u>Carcharhinus limbatus</u>	Black-tip Shark	Black-finned School Shark
<u>C. sorrah</u>	School or Sorrah Shark	
<u>C. amblyrhynchoides</u>	Grey Whaler Shark	
<u>C. dussumieri</u>	Black Spot Shark	
<u>C. melanopterus</u>	Black Fin Reef Shark	
<u>C. amboinensis</u>	Grey Whaler Shark	Milk Shark
<u>C. macrotis</u>	Milk Shark	
<u>Rhizoprionodon acutus</u>	Milk Shark	
<u>Sphyrna blochii</u>	Handle bar Hammerhead	Hammerhead
<u>S. lewini</u>	Scalloped Hammerhead	
<u>S. mokarran</u>	Great Hammerhead	
<u>Carcharhinus cautus</u>	Mangrove Shark	Mangrove Shark

Table 2

Consumer preference for cold- and warm-water shark

Trial	Able to correctly identify odd sample	Preference	
		Cold-water	Warm-water
1	272/380 (72%)	115/272 (42%)	157/272 (58%)
2	293/412 (71%)	136/293 (46%)	157/293 (54%)
3	158/248 (64%)	74/158 (47%)	84/158 (53%)
Total	723/1040 (70%)	325/723 (45%)	398/723 (55%)

Table 3

Consumer perceptions of cold- and warm-water sharks

Gummy shark		School shark	
vs.		vs.	
Grey whaler shark		Hammerhead	
Colour : white	yellow/grey	white	yellow/grey
Texture: moist	flaky	soft, smooth, moist	chewy/flaky
Flavour: bland	salty/(tangy)	bland	fishy, salt

THE MANUFACTURE OF FPC (TYPE B) PRODUCT FORMULATION
USING APPROPRIATE TECHNOLOGY

by

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ABSTRACT

Fish protein concentrate (Type B) was prepared in pre-pilot scale using simple equipment such as mechanical press to remove the liquid and oil after steaming, solar drier for drying, and hand-driven grinder for pulverising. Several species of fish were used such as anchovy, slipmouth, round scad, milkfish, lizard fish, hairtail and frigate mackerel either whole or flesh solution, citrate buffer solution and water for extraction. Citrate buffer-extracted FPC from milkfish was incorporated into local products such as polveron and molido. The fortified products were evaluated and found to be acceptable. FPC tablets and capsules were prepared using a locally fabricated hand tablet machine and capsule filler. Coating of the tablets was done to improve texture, flavour and odour of the tablets.

1. INTRODUCTION

The manufacture of FPC Type A (Finch, 1969) has been known in the past decade to include the use of organic solvents like isopropanol, ethanol and other volatile hydrocarbons. The literature about FPC Type B prepared in Southeast Asia is quite extensive and there are several reviews (Orejano, 1983; Espejo-Hermes, Orejano and Bigueras, 1981; Yamprayoon and Kiatkungwolkrai, 1983) to mention only a few.

This study mainly involves the use of appropriate technology for the local manufacture of FPC Type B in the Philippines. The methods and equipment are simple and highly applicable in a village-scale type of industry. Product formulations using FPC Type B include those of kroepack, fish tablets and capsules and other snack items like polveron, a powdered flour-FPC mixture.

Acceptability studies using the FPC Type B as a daily ingredient in recipes in Filipino diets which were conducted for several months have revealed that the product can be incorporated daily (up to 20 g/day) without any adverse effect.

2. METHODOLOGY

2.1 Preparation of FPC

Several species of fish such as anchovy, slipmouth, frigate mackerel, hairtail, round scad, milkfish and lizard fish were utilized in the FPC manufacture. Two types of minced preparations were used (Figure 1). The FPC samples were prepared by boiling the minced flesh or the whole fish with sodium chloride or citrate buffer (pH 6-7) or water at 95-100°C for 1 h. Partial removal of oil, water and solvent was carried out using a mechanical press (Figure 2). Press cakes were dried using a solar drier (Figure 3a) or an agrowaste drier (Figure 3b). The dried products were stored in bottles. Proximate analysis such as protein by Kjeldahl method (AOAC, 1975), moisture by oven method, fat by Soxhlet method and ash by incineration in a muffle furnace (Trieboald and Aurand, 1963) were conducted.

2.2 Product Formulations

FPC Type B from milkfish (*Chanos chanos*) extracted with citrate buffer (pH = 6.0) was added into local products such as polveron and molido. The fortified products were evaluated using the hedonic scale (1 = extremely like; 9 = extremely dislike). Statistical analysis of the results was done using the t-test and analysis of variance (ANOVA) (Gatchalian, 1981; Larmond, 1970).

Tablets and capsules were prepared from isopropanol-extracted FFC using sharp-nosed shark (*Squalodon palaeopneus* Cuvier). The hand tablet machine and capsule filler are illustrated in Figures 4a, 4b, 4c, and 5, respectively. The tablets were evaluated as to hardness (Lachman, 1976), weight variation, disintegration time (United States pharmacopeia, 1970) and sensory evaluation. Coating of the tablets was done to improve the quality (Regidor, 1980). The coated samples were compared to the uncoated tablets.

3. RESULTS AND DISCUSSION

The proximate composition of several species of fish was determined (Table 1). Fish protein concentrate (Type B) can be prepared using locally fabricated equipment such as a mechanical press (Figure 2) for the removal of fat and moisture and solar and agrovaste driers (Figures 3a and 3b) for drying the pressed cake. The mechanical press was used to reduce the moisture and fat content of the FFC. The moisture ranged from 4.99 to 9.79% while the fat content ranged from 2.93 to 15.84%. The moisture content of the FFC Type B product must not exceed 10% (Windsor, 1977). The fat content, however, of some FFC was higher than the maximum amount allowed (10% for a Type B FFC). The high fat content of some FFC products could be due to the nature of the species used and the type of extractant employed as in the case of milkfish. The FFC produced had a protein content which ranged from 67.09 to 81.50%. The proximate analyses vary with the species and the type of extraction method used.

The FFC (Type B) prepared from milkfish by citrate-buffer extraction was incorporated in two products, polvoron and molido. Polvoron is usually made of flour, butter or margarine, sugar and flavouring. Molido on the other hand, is a product in the Ilocos region which is made of sweet potatoes, sugar and milk. These products are popular among the children although they are deficient in protein. FFC was therefore added to enhance the protein content.

The acceptability test on polvoron showed that the addition of milkfish FFC at 5% level was comparable to that of the control (without FFC) as shown in the t-test where t_c (2.03) was less than the $t = (2.14)$. Based on the remarks given by the panelists, slightly fishy flavour was detected but this did not affect the acceptability of the fortified product.

Results on the acceptability test on molido fortified with various levels (2 and 4%) of milkfish FFC prepared by citrate extraction exhibited difference among the treatments at 1% level of significance (Table 2). The Duncan's multiple Range Test (DMRT) showed that treatment A (control) was not as acceptable as treatment B (2% FFC) and C (4% FFC) at 5% level of significance. The addition of FFC slightly improved the texture of the product as gathered from the remarks of the panelists. Although a slightly fishy taste was detected by the panelists on treatment C, they found it still acceptable.

FFC tablets and capsules were prepared using a punch-type tablet machine (Figures 4a, 4b and 4c) and a fabricated capsule filler (Figure 5), respectively. The punch-type machine is suitable only for small-scale production. Based on the average 12 tablets are produced in 1.5-2.0 min. including the filling of the dies by the material or about 360-484 tablets in 1 h.

The amount of FFC in the tablet can be varied widely. Formulations with 4% binder were friable or easily broken. The hardness of the tablet with 10% binder is acceptable and at the same time has higher protein content than that of the tablets with 15% starch paste (Table 3).

The disintegration time of each formulation determined by using water at 37°C is directly proportional to its hardness ratio. The cohesiveness of the materials in the tablet is measured to a large extent by the hardness ratio and disintegration time.

In order to improve the quality of the tablets, coating with edible shellac was applied. Evaluation of the finished products showed that the tablets measured 5/16 inch in diameter x 2/16 to 3/16 inch thickness with weights ranging from 0.19782 to 0.20846 g in coated tablets and 0.244379 to 0.2471 g for uncoated tablets (Table 4). Based on the protein weight per tablet, uncoated tablets gave much higher weight ranging from 0.14758 to 0.16039 g than coated tablets which gave a protein weight of 0.11524 to 0.13508 g.

The disintegration rate of coated tablets was on the average, 129 sec., much greater than the disintegration time for the uncoated tablets, which was 61 sec. on the average. The coated tablets gave a starting disintegration time between 47-62 sec., lower than the uncoated tablets which began disintegrating 1-2 sec. after its immersion.

As to the sensory evaluation, results proved that coated tablets were superior to uncoated ones. Based on appearance, texture, taste and odour characteristics it was shown that the coated tablets gave a smoother, good appearance and revealed no signs of bitter taste or fishy odour.

The capsule filler developed is simple (Figure 5). On the average 84-98 capsules are prepared in 1 h. Capsules yield higher protein weight ranging from 0.23941 to 0.27515 g (Table 5) than tablets due to the absence of the binder in the former product.

4. CONCLUSION

The manufacture of FPC Type B and various product formulations can be made using appropriate technology. The use of sophisticated equipment required for FPC Type A preparation in developed countries may be replaced by village-type equipment for FPC Type B suited to coastal areas developing countries in Southeast Asia.

5. REFERENCES

- AOAC (Association of the Official Analytical Chemists). Nitrogen determination, improved Kjeldahl method for nitrate free samples. Official methods of analysis of the Association of the Official Analytical Chemists. Washington, D.C., AOAC 12th ed.
- Espejo-Hermes J., F.M Orejana, and C.M Sigueras., Fish protein concentrates (Type B). 1981 Philipp.J.Food Sci.Technol., 5(1)30-7.
- Finch, R., The U.S. fish protein concentrate program. Commer.Fish.Rev., 31(1):25 1969
- Gatchalian, M., Sensory evaluation with statistical analysis. Diliman, Quezon City, University of the Philippines, College of Home Economics, 421 p.
- Lachman, L. et al., The theory and practice of industrial 2nd ed. Pharmacy. Philadelphia, Lea and Febiger, pp. 359-438.
- Larmond, E., Methods for sensory evaluation of food. Ottawa, Canada, Department of Agriculture, 1970 57 p.
- Orejana, F.M., Low-cost fish processing and the use of appropriate technology in the Philippines. 1983 ICLARM Conf. Proc., (8):153-60.
- Rexidor, C., Small-scale preparation of (uncoated/coated) tablets and capsule using fish protein concentrate from sharp-nosed shark (*Scoliodon palasorrah* Olivier). B.S. Thesis, University of the Philippines, Diliman, Quezon City, 37 p.
- Triebold, H. and L.W. Aurand. Food composition and analysis. Princeton, New Jersey, Van Nostrand Co., Inc., pp. 23-4 1963
- United States pharmacopeia, Easton, Pennsylvania Marck Publishing Co., pp. 932-518th ed. 1970
- Windsor, M.L., Fish protein concentrate. Torry advis.Note, (39): 1977
- Yampreyoon, J. and Kiatkungwalkral., Fish protein concentrate for human consumption. FAO Fish.Rep., 1983 (279) Suppl.: 147-51.

Table 1

Proximate analysis of PFC (Type B) from various species of fish extracted with (a) 0.1 M citrate buffer (b) salt solution and (c) water

Species of fish used		Percentage			
Scientific name	Local name	Protein	Moisture	Ash	Fat
<u>Flesh</u>					
a) <u>Decapterus</u> <u>macrodon</u>	galunggong	81.50	8.00	6.30	4.20
<u>Chanos chanos</u>	bangos	76.02	4.83	3.27	15.84
b) <u>Auxis thazard</u>	tulingan	76.96	7.64	3.40	12.00
<u>Whole Fish</u>					
a) <u>Trichiurus</u> <u>haumeia</u>	espada	67.09	9.79	16.68	5.55
b) <u>Leiognathus</u> <u>spp.</u>	sapsap	69.72	6.66	18.18	5.44
<u>Stolephorus</u> <u>spp.</u>	dilis	74.71	5.43	11.30	8.56
c) <u>Saurida</u> <u>tumbil</u>	kalaso	73.53	4.99	18.56	2.93
<u>Leiognathus</u> <u>spp.</u>	sapsap	68.19	7.99	16.60	7.30

Table 2

Analysis of variance and Duncan's Multiple Range Test (DMRT)
results on the acceptability of molido

Source of variance	df	SS	MS	F	F Table	
					5%	1%
Treatment	2	45.092	22.546	14.069*	3.15	4.98
Panelists	21	26.32	1.253	0.780	1.70	2.12
Error	43	68.908	1.602			
Total	66	140.32				

*highly significant

Duncan's Multiple Range Test (DMRT)

Treatment	(A) Control	(B) 2% FPC	(C) 4% FPC
Mean score	2.409	3.136	4.409
P	2	3	
rp (5%)	2.93	3.08	
Rp	0.7095	0.83	

Statistical findings:

A BC

Note: Samples connected by line not significantly different, and
vice versa

Table 3

Average weight and protein content of the tablets using isopropanol-
extracted FPC from sharp-nosed shark (*Scoleciodon paiaorrah* Cuvier)

% Starch paste (binder)	Tablet average weight (mg)	Protein content (mg)	Protein/tablet (%)
4	229.40	153.85	67.06
10	211.25	135.00	63.91
15	207.60	125.80	60.60

Table 4

Weight of protein per uncoated and coated tablet with isopropanol-extracted FPC from sharp-nosed shark (*Squalodon palaeorrah* Cuvier)

Tablet	Samples	Weight of tablet (mg)	Weight of FPC in tablet (mg)	Weight of protein/ tablet (mg)
Coated	1	197.82	158.26	128.19
	2	177.84	142.27	115.24
	3	208.46	166.77	135.08
Uncoated	1	243.51	195.03	157.98
	2	247.51	198.01	160.39
	3	243.18	194.54	157.58

Table 5

Weight of protein per capsule filled with isopropanol-extracted FPC from sharp-nosed shark (*Squalodon palaeorrah* Cuvier)

Samples	Weight of filled capsule (mg)	Weight of FPC/ capsule (mg)	Weight of protein/ capsule (mg)
1	482.15	343.94	275.15
2	452.33	314.12	251.29
3	437.48	299.27	239.41

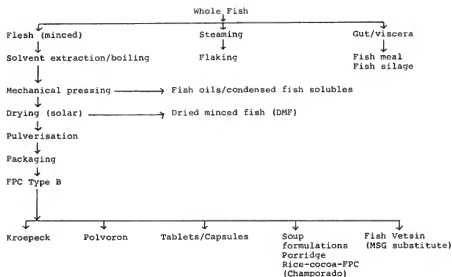


Figure 1 Flow diagram of FPC manufacture of formulated products

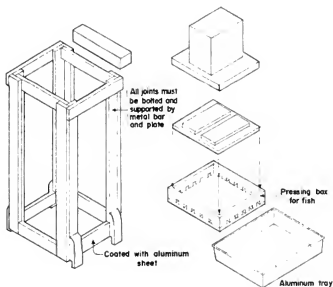


Figure 2 Mechanical press

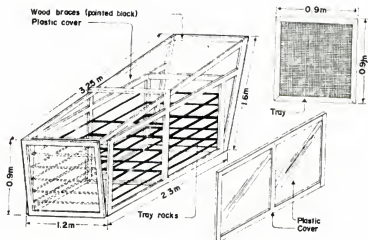


Figure 3a Solar drier

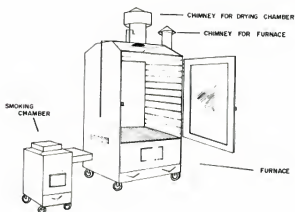


Figure 3b Solar agro-waste multipurpose (S.A.M.) drier/smoker

HAND TABLET MACHINE

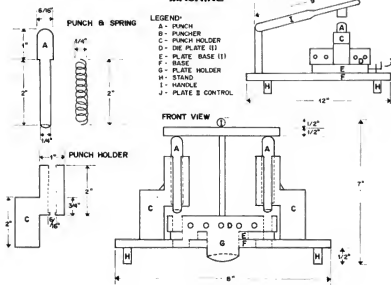


Figure 4a Hand tablet machine

DIE PLATES (TOP VIEW)

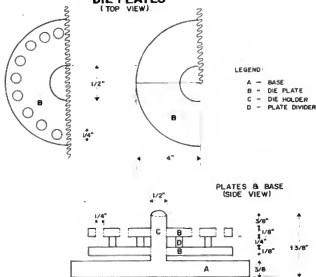


Figure 4b Hand tablet machine

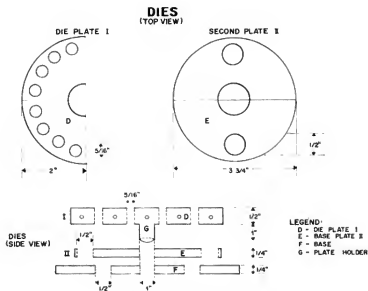


Figure 4c Hand tablet machine

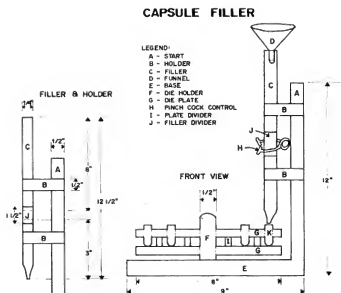


Figure 5 Capsule filler

UTILIZATION OF FISH BY-CATCH FOR FISH-BALL MANUFACTURE

by

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ABSTRACT

Investigations were undertaken with marine by-catch and freshwater fish to determine (i) the relationship between the freshness index of the raw material and functional properties of the meat; (ii) the effect of leaching, addition of polyphosphate, setting time and species on fish-ball quality; and (iii) the quality of fish balls from a mixture of minced by-catch and commercial species. Results showed that the storage life of by-catch species in ice was 9-15 days and freshwater fish 16-18 days. The cohesiveness of fish balls from by-catch was acceptable from 10-12 days while tilapia was satisfactory after 23 days, and common carp 36 days. Good quality fish balls could be made from meat leached in diluted salt solutions and heat treated at 40°C for 20 min. Leaching was found to improve colour and gel texture but varied with species. Gel strength, colour and flavour of minced by-catch and commercial species were evaluated and grouped. Threadfin bream and goat fish from the by-catch had gel strengths in the range of the preferred commercial species, flatfish and ribbon fish. The colour of pony fish from the by-catch was as good as flatfish. It is possible to substitute minced by-catch as part of the raw material for non-leached fish balls, at a maximum rate of 50% or 75% for predominantly palagic and demersal species, respectively.

1. INTRODUCTION

By-catch in Thailand includes a wide variety of small demersal fish. Kuanthanom (1980) investigated the composition of by-catch and found juveniles of commercially important species belonging to 36 families, and individuals of commercially less important species belonging to 35 families. The latter group comprised 32 demersal fish families and three invertebrate families. Approximately 800 000 t of by-catch are landed annually and used mainly for fishmeal production. The quality of by-catch is generally low because of poor onboard handling and lack of chilling facilities. However, the quality of the last day's catch is generally better. Utilisation of this fish as food, such as fish-ball manufacture, will increase the potential of the fisheries resources for human consumption and give added value to the raw material. It is estimated that over 3.7 million fish balls are produced per day in Bangkok which requires an equivalent of 4.5 t of whole fish (Anon., 1978). The traditional sources of fish for fish-ball manufacture can be divided into two groups: low cost fish such as bigeye, lizardfish, threadfin breams, barracuda, red fish and high cost fish like flatfish, dorab and sea eel.

The general objectives of the present study were to determine the characteristics of local by-catch as raw material for fish-ball manufacture; and to develop staff capability in understanding fish raw material, quality characteristics and testing methods as a basis for future process development work and fish utilization in Thailand. The fish processing section under the Fish Technology Development Division, Department of Fisheries, Ministry of Agriculture and Co-operatives was assigned to undertake this project which is sponsored by IDRC.

1.1 Specific Objectives

- (i) To study the effect of ice storage on functional properties of the fish meat.
- (ii) To evaluate the effects of species, leaching, addition of polyphosphate and setting time on fish-ball quality.
- (iii) To compare the properties of fish balls prepared from a mixture of minced by-catch and commercial species.

2. MATERIALS AND METHODS

2.1 Raw Material

The fish used for these studies were divided into two groups:

- (i) by-catch species
- (ii) commercial species

The more common by-catch species used were threadfin braams (Nemipteridae), sola (Synodontidae), flat-head (Platycephalidae), pony fish (Leiognathidae), goat fish (Mullidae) and lizardfish (Synodontidae) and the commercial species used were threadfin bream (Nemipteridae), bigeye, sole, tilapia (*T. nilotica*) and common carp (*C. gomionotus*).

2.2 Chemical Analysis

Fat, protein, ash and moisture were determined using the official methods of AOAC (1980). Total volatile nitrogen (TVN) was determined by the Conway method (Uchiyama, 1978). Salt soluble protein (SSP) was extracted from a mixture containing 5% salt solution and 100 g ica (mixture equivalent to 0.854 M NaCl and 0.02 M NaHCO₃, Umemoto, 1966). Gel strength was measured using a Rheometer (Suzuki, 1981). A folding test was established to measure "flexibility" of a 3-mm disc of fish ball when it was folded into halves and quarters (Suzuki, 1981).

2.3 Sensory Analysis

Sensory analysis of raw fish was carried out by six trained panelists and expressed in terms of appearance, texture and flavour using the scorecard in Table 1.

Sensory evaluation of fish balls was carried out using the scorecard presented in Table 2.

2.4 Effect of Ice Storage on Functional Properties of the Meat

Commercial marine and freshwater fish were obtained from trawlers and local markets, and stored in ice during the experiment. Cohesiveness was measured by a sensory evaluation of the fish ball, which were produced by the scheme outlined in Chart 1.

2.5 Comparative Study of Species, Leaching, Addition of Polyphosphates and Setting Time on Properties of Fish Balls

Five common species of by-catch were chosen: pony fish, threadfin braams, lizardfish, flatfish and flathead and two species of freshwater fish, tilapia and common carp. Freshness, weight and size were determined prior to preparation for analysis. Minced fish was prepared from each species by a deboning machine. Leached meat was prepared by washing the minced flesh at about 10°C with 0.2% and 0.3% salt solution for 15 min consecutively. Excess water was removed using a screw press. Combinations of leaching, non-leaching and addition of polyphosphates to evaluate the effect on colour and gel texture of fish balls was carried out (Chart 2). For each minced fish species, pH, total volatile base, salt soluble protein, moisture, protein, fat and ash were determined. For the determination of optimum setting time, the leaching method with the addition of polyphosphates was used and properties of fish balls for setting times of 20, 40 and 60 min, respectively, were compared.

2.6 Comparative Study of Fish-Ball Properties from Mixed Marine By-catch Species and the Commercial Species (Industrial Scale)

A semi-commercial test on the quality of fish balls using minced by-catch mixed with some commercial species was carried out. Sola, dorab and bigeye were bought in fillet form and kept in ice prior to processing. By-catch was taken from the top of the whole catch which had been caught two or three days previously from single commercial trawler. All fish were washed in chilled water, headed and gutted and meat separated using a meat-bone separator. All minced meat was well kept in ice until used. Fish balls were prepared in 60-kg batches by mixing by-catch with sola, dorab, bigeye 3:1:5 at 252-75% using regular factory process.

3. RESULTS AND DISCUSSION

3.1 Effect of Ice Storage on Functional Properties

The sensory assessment score for each species is shown in Tables 3-9. Quality gradually decreased with iced storage times.

The results of the relationship between freshness of the raw material after iced storage and functional properties of the fish balls are shown in Table 10. The cohesiveness of fish balls produced from tilapia and carp was acceptable up to the 16th and 33rd days of iced storage, respectively. The other commercial species had much shorter storage times. The cohesiveness of products from by-catch was ranked as acceptable up to 10-12 days with the exception of lizardfish (Table 10). The flavour was judged to be acceptable for all fish except sole which was very fishy and lizardfish which had a bland taste. Sensory scores and folding tests showed a high correlation ($p = 0.05$) with the gel-forming abilities of the minced fish. These results show the amount of time fish can be stored in ice before processing.

3.2 Comparative Study of Species, Leaching, Addition of Polyphosphate and Setting Time on Properties of Fish Balls

The species, size, yield, freshness and proximate composition of fish used in this experiment are shown in Table 11. The size of marine species used was very small (approximately 10-cm length) and the freshness was not really good (Average TVB-N = 16 mg/100 g).

From Trial 1, the effect of species, leaching and addition of polyphosphate on gel texture and some other properties are shown in Table 12. The difference in cohesiveness among species is significant. Although the sensory score for cohesiveness of fish balls from non-leached minced fish and leached minced fish varies, higher sensory scores were recorded for fish balls made from leached minced fish. The difference of cohesiveness between leached and non-leached within the same species does not appear to be significant. Absence or presence of polyphosphate affects cohesiveness more than the application of the leaching technique.

From Trial 2, the effect of setting time on gel texture and some other properties are shown in Table 13. Little difference in elasticity and gel strength of fish balls was demonstrated for setting times of 20 min, 40 min and 60 min. Nevertheless, higher sensory scores and gel strength were found for setting times of 20 min than those of 40 min and 60 min, except for tilapia.

The results of the rheometer tests show that tilapia and flathead had good gel-forming ability while other species had good gel strength (Table 13).

Minced fish obtained from a mechanical deboner consists of blood and dark muscle resulting in fish balls with a dark and unacceptable colour (Howgate, 1976). When 0.2% and 0.3% salt solution were used to wash this flesh, the fish mince was whiter. Fish balls made from leached minced fish not only had a good white colour but also good gel strength. High correlation was shown between the instrumental measurement of gel strength and the sensory evaluation on elasticity.

A leaching method has, therefore, been elaborated to improve colour and promote cohesiveness. However, the final product lacks the natural flavour of fish. Normally, fairly fresh by-catch had a sweet and slightly meaty flavour. As the fish deteriorated, the sweetness and meatiness decreased and loss of yield were considerable (yield of leached meat was approximately 30%).

The gel strength of the products can be improved only if the setting condition is correct. The results seem to suggest that heating fish balls at 40°C for 20 min, 40 min and 60 min is effective.

3.3 Comparative Study of the Properties of Fish Balls Prepared From Minced Marine By-catch Species and the Commercial Species (Industrial Scale)

The properties of fish balls from different ratio of mixed commercial fish with by-catch are shown in Table 14. Fish balls from minced by-catch had gel elasticity and colour significantly different from mixed commercial minced meat. Minced by-catch could be used as part of raw material to substitute up to 25%-75% of commercial fish. The degree of substitution may depend on the composition of by-catch itself. From Trial 1, which consisted of 99.9% demersal species, 75% of minced by-catch could be used to replace commercial grade fish. Most of pelagic species had black meat which may interfere with gel elasticity and colour of fish balls. In Trial 2, which consisted of pelagic species (63.2%), the maximum degree of substitution was 50% which resulted in no significant difference in quality with the commercial fish balls. All results were shown in Charts 3 and 4.

4. CONCLUSIONS AND RECOMMENDATIONS

Our results show that by-catch species have shorter storage lives in ice (9-15 days) when compared to freshwater fish (16-18 days). The cohesiveness of fish balls from by-catch was acceptable from 10 to 12 days, while tilapia and carp were satisfactory after 23 and 36 days, respectively. Good quality fish balls can be manufactured from leached minced fish with a setting time of 20 min at 40°C. Leaching improved the colour and gel texture, but varied with species.

Minced by-catch can be substituted as part of the raw material for non-leached fish balls, at a maximum rate of 50% and 75% for predominantly pelagic and demersal species, respectively.

Following a workshop on fish-ball production from by-catch at the FTDD, fishermen and fish-ball manufacturers express willingness to cooperate in substituting by-catch for commercial species.

FTDD will act as the information centre in coordinating this project and will continue to work on the improvement of by-catch yields.

5. REFERENCES

- AOAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1980 AOAC, 13th ed.
- Howgate, P., The sensory properties of minced cod and herring. In Processings of the Conference 1976 on the production and utilisation of mechanically recovered fish flesh (Minced flesh) edited by J. Keay. Aberdeen, Torry Research Station, pp. 49-53
- Kauntanon, N., Trash fish composition and utilisation of commercial single trawlers in Gulf of Thailand in 1978. Rep.Demersal Fish Dep.Fish.,Bangkok, (1)
- Suzuki, T., Fish and krill proteins: processing technology. London, Applied Science Publishers, 1981 Ltd., 260 p.
- Uchiyama, H., Analytical methods for estimating freshness of fish. Phrapradaeng, Samut Prekan, 1978 Thailand, Training Department Asian Fisheries Development Center
- Umemoto, S., A modified method for estimation of fish muscle protein by Biuret method. Bull.Jap. Soc.Sci.Fish., 32:427-35

Table 1

Fresh fish assessment

						Name	_____
						Date	_____
I. APPEARANCE							
Eyes	5	4	3	2	1		
	full, black pupil	slightly sunken	greyish pupil	completely sunken	yellow-brown, heavy slime		
Gills	bright red or pink	dark red	greyish white slime	bloody, discoloured	yellow or brown slime		
Skin	glossy, smooth	loss of sheen	dull, greyish slime	discoloured slime	thick yellow-brown slime		
II. ODOUR							
	5	4	3	2	1		
	fresh (smell of sea)	neutral	fishy smell	slightly ammoniacal smell	off odour sulphidic		
III. FLESH							
Texture	5		3		1		
	firm, elastic		firm, not elastic, slightly gaping		soft, doughy		
Appearance	glossy, translucent, pinkish sheen		slightly red, opaque		bloody appearance, dark red, brown or yellow discolouration		
IV. BELLY FLESH							
Texture	5		3		1		
	firm		slight breakage of skin		digested, broken down		
Appearance	pink sheen		opaque, greyish flesh		yellow, brown discolouration		
V. REMARKS							

Table 2

Sensory evaluation of fish balls

						NAME _____
						DATE _____
I. APPEARANCE						
Colour	5	4	3	2	1	
	white	pale yellow or grey	dark grey		brown	
	white		grey		brown or discoloured	
Gloss	shiny				dull	
Surface	smooth		slightly porous		rough	
Imperfection (skin, blood, scale, etc.)	low				high	
II. TEXTURE						
Initial-elastic	10		5		1	
mouth sensation	springiness				crumbly	
Initial-hardness	5	4	3	2	1	
	soft				hard	
Particle characteristic	smooth				rough	
Succulence	moist				dry	
III. FLAVOUR						
Overall flavour	5	4	3	2	1	
	fresh, shellfish- like	neutral	slightly fishy	strong fishy/ oily	unpleasant or stale	
Taste (comment on taste as salty, sweet, bland, etc.)						
IV. REMARKS						

Table 3

Sensory assessment score of fish-ball properties and folding test of threadfin breams (average length 17.85 cm)

Days in ice storage	Cohesiveness	Folding test
1	6.3 \pm 0.56	AA
2	8.1 \pm 0.41	AA
3	7.0 \pm 0.61	A ⁻
4	6.5 \pm 0.41	B ⁺
5	7.6 \pm 0.99	B
6	5.6 \pm 0.63	C ⁺
7	6.0 \pm 0.51	C ⁺
8	7.2 \pm 0.58	B ⁻
9	7.6 \pm 0.99	B ⁺
10	6.6 \pm 0.80	AA
11	5.0 \pm 0.65	C

Table 4

Sensory assessment score of fish-ball properties and folding test of threadfin breams (average length 11.84 cm)

Days in ice storage	Cohesiveness	Folding test
2 ½	7.93 \pm 0.65	A ⁻
4	7.00 \pm 0.56	B ⁺
6	8.50 \pm 0.95	A
9	5.28 \pm 0.50	B ⁺
10	3.60 \pm 0.23	D ⁺
11	3.57 \pm 0.73	D
13	3.18 \pm 0.33	D
14	7.30 \pm 0.41	D ⁺

Table 5
Sensory assessment score of fish-ball properties and folding test of flatfish (sole)

Days in ice storage	Appearance	Texture	Flavour	Average	Remarks	
					Cohesiveness	Folding test
1	2.3 ± 0.32	2.8 ± 0.10	2.83 ± 0.24	2.80 ± 0.23	7.0 ± 0.00	A ⁺
5	2.61 ± 0.80	3.09 ± 0.24	2.83 ± 0.24	2.84 ± 0.20	6.66 ± 1.18	A ⁻
9	3.29 ± 0.13	2.56 ± 0.07	1.0 ± 0.00	2.28 ± 0.95	7.5 ± 0.50	B
12	3.53 ± 0.26	3.07 ± 0.15	1.33 ± 0.47	2.64 ± 0.95	3.16 ± 1.03	C ⁻
15	2.97 ± 0.38	2.65 ± 0.56	1.13 ± 0.32	2.25 ± 0.80	5.0 ± 1.06	C
19	2.88 ± 0.26	2.62 ± 0.44	1.0 ± 0.00	2.17 ± 0.33	4.0 ± 0.82	C

Table 6
Sensory assessment score of fish-ball properties and folding test of flathead

Days in ice storage	Appearance	Texture	Flavour	Average	Remarks	
					Cohesiveness	Folding test
1	4.43 ± 27	3.69 ± 0.22	4.00 ± 0.90	4.04 ± 0.36	6.5 ± 0.00	A
5	3.86 ± 0.06	3.75 ± 0.19	2.5 ± 0.50	3.37 ± 0.62	6.5 ± 0.00	D
8	3.60 ± 0.16	3.5 ± 0.00	2.50 ± 0.50	3.22 ± 0.51	6.0 ± 0.00	D
11	3.85 ± 0.36	3.04 ± 0.15	1.66 ± 0.94	2.85 ± 0.90	2.3 ± 1.25	D
15	3.45 ± 0.21	3.11 ± 0.33	1.5 ± 0.5	2.69 ± 0.85	1.88 ± 1.24	D
20	3.66 ± 0.47	3.19 ± 0.31	1.0 ± 0.00	2.61 ± 1.16	1.83 ± 0.85	D

Table 7
Sensory assessment score of fish-ball properties and folding test of lizardfish

Days in ice storage	Appearance	Texture	Flavour	Average	Remarks	
					Cohesiveness	Folding test
1	3.24 ± 0.33	2.44 ± 0.44	4.30 ± 0.51	3.33 ± 0.75	1.31 ± 0.22	C ⁻
5	1.85 ± 0.39	1.85 ± 0.50	2.33 ± 0.47	2.01 ± 0.23	1.0 ± 0.00	C
8	3.20 ± 0.52	2.79 ± 0.65	3.40 ± 0.47	3.14 ± 0.22	4.67 ± 1.97	C ⁻
11	2.14 ± 0.38	2.13 ± 0.50	2.28 ± 0.23	2.18 ± 0.06	1.25 ± 0.36	C ⁻
15	2.97 ± 0.33	2.25 ± 0.45	2.28 ± 0.47	2.50 ± 0.29	1.67 ± 0.82	D
20	3.52 ± 0.36	2.19 ± 0.31	1.92 ± 0.38	2.55 ± 0.61	0.94 ± 0.17	D

Table 8
Sensory assessment score of fish-ball properties and folding test of tilapia

Days in ice storage	Appearance	Texture	Flavour	Average	Remarks	
					Cohesiveness	Folding test
1	3.98 ± 0.39	4.07 ± 0.32	4.00 ± 1.00	4.01 ± 0.40	7.00 ± 0.00	AA
4	3.69 ± 0.21	3.58 ± 0.32	3.38 ± 0.65	3.48 ± 0.13	3.37 ± 0.41	C
7	3.72 ± 0.14	3.63 ± 0.28	3.38 ± 0.65	3.58 ± 0.14	6.50 ± 0.50	B
10	3.81 ± 0.13	3.59 ± 0.17	3.25 ± 0.25	3.55 ± 0.25	7.18 ± 0.44	B ⁺
12	3.71 ± 0.09	3.50 ± 0.22	3.13 ± 0.41	3.45 ± 0.24	7.13 ± 0.22	AA
14	3.79 ± 0.90	3.38 ± 3.45	3.13 ± 0.41	3.43 ± 0.27	7.50 ± 0.35	AA
16	3.78 ± 0.90	3.77 ± 0.16	3.00 ± 0.35	3.52 ± 0.37	6.25 ± 0.25	C
20	3.66 ± 0.10	3.58 ± 0.22	2.75 ± 0.56	3.88 ± 0.41	6.30 ± 0.41	C ⁻
23	3.56 ± 0.26	3.60 ± 0.11	2.88 ± 0.41	3.88 ± 0.33	5.25 ± 0.56	C

Table 9
Sensory assessment score of fish-ball properties and folding test of common carp

Days in ice storage	Appearance	Texture	Flavour	Average	Remarks	
					Cohesiveness	Folding test
1	4.31 ± 0.01	3.85 ± 0.32	3.88 ± 0.50	4.01 ± 0.27	7.75 ± 0.31	AA
5	3.99 ± 0.26	3.67 ± 0.28	3.75 ± 0.33	3.00 ± 0.20	8.00 ± 0.20	AA
8	4.07 ± 0.30	3.44 ± 0.49	3.75 ± 0.43	3.82 ± 0.25	7.75 ± 0.23	AA
11	3.83 ± 0.17	3.35 ± 0.33	3.75 ± 0.09	3.64 ± 0.13	7.55 ± 0.35	AA
13	3.81 ± 0.35	3.39 ± 0.22	3.87 ± 0.51	3.69 ± 0.47	7.50 ± 0.27	AA
15	3.79 ± 0.22	3.45 ± 0.29	3.50 ± 0.35	3.58 ± 0.55	6.88 ± 0.15	A ⁺
18	3.38 ± 0.41	3.45 ± 0.25	3.33 ± 0.22	3.88 ± 0.56	7.00 ± 0.17	A ⁺
20	3.41 ± 0.08	3.42 ± 0.32	3.33 ± 0.10	3.38 ± 0.32	6.66 ± 0.18	A ⁺
22	3.50 ± 0.24	3.48 ± 0.71	3.00 ± 0.43	3.32 ± 0.17	7.00 ± 0.21	A ⁺
26	3.53 ± 0.19	3.48 ± 0.55	2.83 ± 0.33	3.20 ± 0.20	6.10 ± 0.40	C
28	3.53 ± 0.20	3.36 ± 0.67	3.00 ± 0.35	3.30 ± 0.31	5.50 ± 0.15	C ⁻
29	3.29 ± 0.80	3.49 ± 0.35	3.00 ± 0.47	3.26 ± 0.19	6.50 ± 0.53	A ⁻
32	3.11 ± 0.75	3.33 ± 0.22	2.33 ± 0.35	2.92 ± 0.25	6.66 ± 0.34	B
34	3.11 ± 0.25	3.27 ± 0.67	2.16 ± 0.45	1.84 ± 0.19	6.50 ± 0.11	B
30	3.13 ± 0.15	3.19 ± 0.33	2.16 ± 0.55	2.80 ± 0.20	5.80 ± 0.77	B ⁻

Table 10

Critical points of raw materials and the produced fish balls (days)

Species	TVB 25 mg (g)	Organoleptic			Folding test c/AA 3/5 score
		Freshness 2.5/5 score	Cohesiveness 5/10 score	Flavour 3/5 score	
Threadfin bream (8-20 October 1980)	9	9	12	a/	12
Threadfin bream (10-22 November 1980)	14	9	10	a/	10
Flathead (12-30 March 1981)	15	12	10	5	5
Flatfish (sole) (12-30 March 1981)	15	12	12	1	12
Lizardfish (18 June - 6 July 1981)	19	15	1	1	1
Tilapia (17 February - 9 March 1981)	23	16	23	16	20
Common carp (24 April - 29 May 1981)	29	18	36	33	26

a/ The critical point at the last day of the experiment was not met

Table 11
Species, size, yield of minced meat, pH, freshness, salt soluble protein and proximate composition of fish used in the investigation (batch weight is 4-5 kg)

Species	SL (cm)	SW (g)	Yield (%)	pH	TVB mg/100 g	Freshness score 1-5	SSP mg/g	N (%)	P (%)	Fat (%)	Ash (%)
Pony fish	8.73	10.60 (94)	30.8	6.0-6.46 (6.23)	8.21-12.37	3.2-3.4 (3.3)	21.3	82.33	15.9	0.75	1.15
Threadfin bream	11.68	23.19 (43)	41.67	6.3-6.6 (6.35)	6.28-19.21	3.5-4 (3.75)	27.64	80.72	19.1	0.29	1.44
Lizardfish	10.86	7.59 (132)	45.0	6.0-6.7 (6.35)	9.73-32.48	2.0-3.9 (2.95)	22.99	84.72	15.07	9.458	1.34
Flatfish (sole)	9.22	6.09 (164)	40.4	5.2-6.7 (6.45)	12.67-24.16	3.2-3.5 (3.35)	26.78	82.17	16.06	0.63	1.09
Flathead	11.57	10.65 (94)	43.2	6.2-6.38 (6.29)	12.67-20.98	3.83-3.9 (3.87)	22.97	82.70	17.06	0.14	1.22
Goat fish	12.21	32.10 (31)	43.8	6.3-6.64 (6.38)	12.81-20.63	3.56-4.5 (2.80)	26.06	76.95	10.72	0.29	1.44
Tilapia	17.75	350.0 (3)	40.1	6.0-6.3 (6.15)	8.9-15.6	4.5-5 (4.75)	24.84	77.4	17.10	1.8	1.06
Carp	19.11	389.0 (3)	35.3	6.1-6.4 (6.25)	9.64-19.73	4.0-5 (4.5)	23.12	74.1	18.19	2.6	1.2

N.B.: SL = standard length; SW = body weight; TVB = total volatile nitrogen; SSP = salt soluble protein; N = moisture; P = protein

Table 12
Effect of leaching and addition of polyphosphate on gel strength and some other properties

Species	Replication	NL			NLP						
		Colour	Flavour	Elasti-city	Gel Strength (g-cm)	Folding test	Colour	Flavour	Elasti-city	Gel Strength (g-cm)	Folding test
Pony fish	1	3.1	3.4	8.8	-	AA	2.7	3.6	9.12	-	AA
	2	3.5	3.83	5.0	-	AA	3.9	3.9	3.9	-	AA
Threadfin bream	1	3.8	3.4	2.4	-	B	3.0	3.5	8.1	-	A
	2	3.5	2.8	3.5	-	C	3.0	2.0	8.4	-	AA
Lizard fish	1	2.8	2.9	1.6	-	D	2.6	2.9	1.5	-	D
	2	2.5	2.5	1.9	-	D	2.5	2.5	1.8	-	D
Flat fish	1	3.56	3.1	8.76	-	AA	3.4	3.5	8.25	-	AA
	2	3.4	3.07	7.67	168	AA	3.67	3.5	8.0	180	AA
Plathead	1	3.86	3.88	5.0	-	A	3.9	2.88	5.0	-	A
	2	4.07	3.0	6.63	185	AA	3.57	3.63	7.5	233	AA
Goat fish	1	2.50	3.6	6.8	-	AA	3.3	3.8	8.75	-	AA
	2	2.17	3.3	8.25	-	AA	2.23	2.3	8.88	-	AA
Tilapia	1	2.2	3.0	3.75	73.6	AA	2.3	3.0	4.7	72	AA
	2	2.0	3.4	5.3	218	AA	1.8	3.4	5.16	263	AA
Common carp	1	3.58	3.5	3.92	101	A	3.92	3.5	4.25	138	AA
	2	2.28	3.25	2.0	88.6	A	2.12	3.58	3.67	146	AA

NL = Non-leaching

NPL = Non-leaching + polyphosphate

Table 12 (continued)

Species	Replication	L					LP				
		Colour	Flavour	Elasti-city	Gel Strength (g-cm)	Folding test	Colour	Flavour	Elasti-city	Gel Strength (g-cm)	Folding test
Pony fish	1	3.42	3.7	9.0	-	AA	3.56	3.8	9.38	-	AA
	2	3.93	3.63	5.17	-	AA	4.33	3.7	8.5	-	AA
Threadfin bream	1	4.92	4.7	8.4	-	AA	4.96	4.2	9.8	-	AA
	2	4.3	2.4	8.0	-	AA	4.7	2.6	8.6	-	AA
Lizard fish	1	4.8	3.4	4.54	-	C	4.8	3.6	6.6	-	AA
	2	4.5	3.0	5.4	-	C	4.8	3.0	6.1	-	AA
Flat fish	1	3.94	3.3	7.76	-	AA	4.1	3.3	8.76	-	AA
	2	4.5	3.17	9.0	263	AA	5.0	4.17	9.0	421.9	AA
Flatfish	1	4.63	3.25	8.38	-	AA	4.63	3.25	2.13	-	C
	2	4.76	3.75	8.25	243	AA	4.88	3.75	3.75	296.9	AA
Goat fish	1	4.06	3.8	8.25	-	AA	4.06	3.6	9.2	-	AA
	2	3.5	3.3	1.15	-	A	3.77	3.3	6.8	-	AA
Tilapia	1	4.1	4.3	8.8	142.5	AA	4.3	4.30	8.75	170	AA
	2	4.2	4.0	9.5	533	AA	4.08	4.75	9.9	533	AA
Common carp	1	4.83	3.9	5.7	181	AA	4.42	4.0	7.0	215.5	AA
	2	3.67	3.5	5.16	151	AA	3.41	3.5	5.83	152	AA

L = Leaching

LP = Leaching + polyphosphate

Table 13
Effect of setting time (20, 40 and 60 min) on gel-forming ability of fish

Species	Replication	20 min				40 min					
		Colour	Flavour	Elasticity	Gel Strength	Folding test	Colour	Flavour	Elasticity	Gel Strength	Folding test
Pony fish	1	3.88	3.4	1.9	-	B	3.88	3.6	1.8	-	C
	2	2.9	2.6	5.4	-	A	2.9	2.8	4.2	-	B
Threadfin bream	1	4.8	3.8	9.1	201	AA	4.6	3.41	7.0	72.5	AA
	2	4.25	4.0	7.8	112.5	AA	4.0	3.8	6.5	88	AA
Lizard fish	1	3.50	2.5	2.08	22.5	D	3.58	2.33	1.08	29.7	D
	2	4.5	4.13	6.25	120	AA	4.5	4.13	5.63	67.7	A
Flat fish	1	5.0	4.16	9.0	422	AA	5.0	4.16	9.0	393	AA
	2	4.83	4.08	8.41	305.6	AA	4.75	4.08	8.08	265	AA
Flathead	1	4.88	3.75	9.30	297	AA	5.0	4.0	9.25	253	AA
	2	4.43	3.75	7.75	226.9	AA	4.18	3.33	7.5	189	AA
Goat fish	1	3.04	3.6	8.42	-	AA	3.0	3.6	7.9	-	AA
	2	3.5	3.7	7.93	181.3	AA	3.26	3.63	7.63	170	AA
Tilapia	1	4.83	3.67	8.7	572	AA	4.83	1.3	8.0	564	AA
	2	4.92	4.08	8.83	563	AA	4.83	4.08	9.08	597	AA
Common carp	1	4.42	3.83	7.0	215	AA	4.25	3.8	7.16	218	AA
	2	4.6	3.6	8.4	310	AA	4.53	3.31	8.1	280	AA

Table 13 (continued)

Species	Replication	60 min				Folding Test
		Colour	Flavour	Elasticity	Gel strength	
Pony fish	1	2.28	3.6	1.45	-	C
	2	2.9	2.6	2.0	-	C
Threadfin bream	1	4.6	3.25	6.8	37.4	A
	2	4.3	3.8	5.0	85.27	A
Lizardfish	1	3.58	2.08	1.0	20.3	D
	2	4.5	3.75	5.38	71.1	A
Flat fish	1	5.0	4.33	8.33	358.3	AA
	2	4.75	4.08	7.67	263	AA
Flathead	1	4.88	3.63	8.75	217	AA
	2	4.18	3.83	7.27	185	AA
Goat fish	1	2.87	3.6	7.83	-	AA
	2	3.04	3.12	7.44	141	AA
Tilapia	1	4.71	3.6	8.41	560.8	AA
	2	4.84	3.52	5.58	608	AA
Common Carp	1	4.13	3.83	7.08	224	AA
	2	4.32	3.44	7.19	278	AA

Table 14
The properties of fish balls differentiated by scores of cohesiveness, colour and flavour at different mixed by-catches^{a/}

Experiment	Cohesiveness (scores) Trial I Trial II	Colour (scores) Trial I Trial II	Flavour (scores) Trial I Trial II	Appearance (scores) Trial I Trial II		Gel-strength (g-cm) Polding test
				Outer	Inner	
I Commercial : mixed by- catch 3 : 1	9.33 8.92	4.2 4.25	3.3 2.2	3.4 3.7	3.5 3.75	383.95, 229.77 AA,AA
II Commercial : mixed by- catch 1 : 1	9.08 8.5	4.0 3.67	3.4 2.5	3.9 3.7	3.6 3.5	198.77, 232.04 AA,AA
III Commercial : mixed by- catch 1 : 3	8.63 6.92	3.83 2.83	3.4 2.75	3.8 3.9	3.6 3.5	153.25, 98.01 AA,AA
IV Commercial : mixed by- catch 0 : 1	5.75 1.67	3.08 2.33	3.3 2.5	3.4 3.5	3.2 3.25	78.68, 25.33 B,D

a/ Mean of six sensory evaluation scores

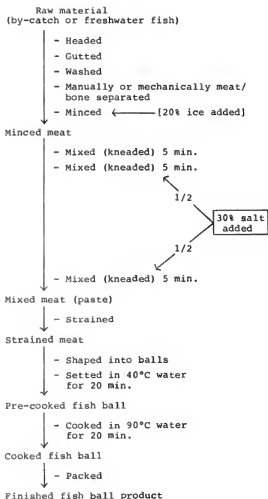


Chart 1 Fish-ball process model

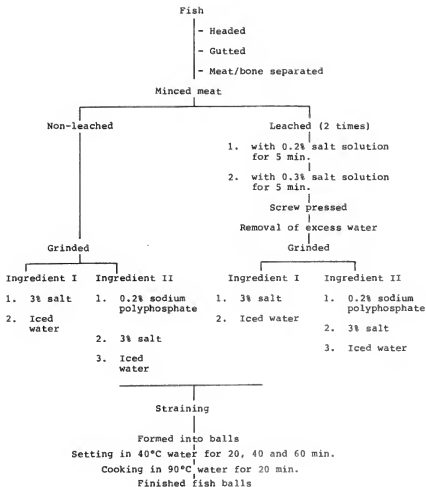


Chart 2 Fish balls manufactured by different treatments

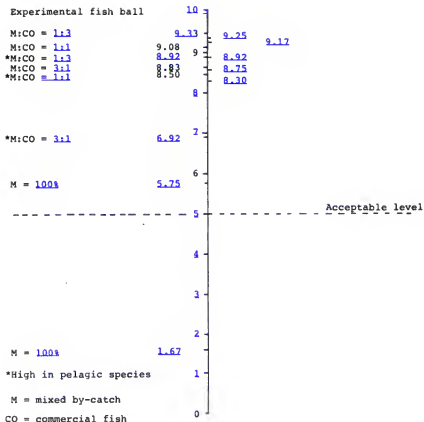


Chart 3 Gel elasticity of fish ball from minced by-catch mixed with commercial fish at different proportions in comparison with locally purchased fish ball

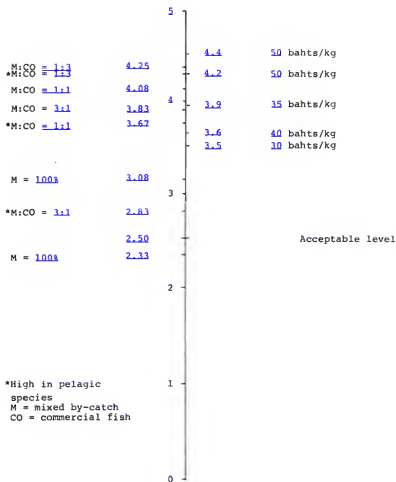


Chart 4 Colour of fish ball from minced by-catch and commercial fish at different proportions in comparison with locally purchased fish ball

STORAGE STUDIES OF FORMULATED PRODUCTS FROM MINCED SPRATS
(*Sprattus sprattus*)

by

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ABSTRACT

Whole unviscerated sprats (*Sprattus sprattus*) were minced in a commercial flash/bone separator. Washed and unwashed mince was incorporated into fish balls and fish sausage as major and minor ingredient, respectively, and stored both vacuum and non-vacuum packed. Nutritional, microbiological and sensory properties were determined and all products had acceptable chilled and frozen shelf lives. Products from washed mince were found to be more acceptable by taste panelists but had higher production costs.

1. INTRODUCTION

In many developing countries, a large proportion of the population are inadequately nourished on account of existing diets being frequently nutritionally unbalanced and, in particular, there is protein shortage. An adequate and balanced protein intake can often be obtained by the addition of relatively small amounts of animal protein to diets in which plant-derived foodstuffs predominate.

A large quantity of many types of protein are wasted today in terms of human consumption because conventional processing methods are inadequate to extract them (Wallyn, 1976). A vast amount of underutilized species of fish are not marketable as whole fish or fillets because of their size, high proportion of bones and consumers' unfamiliarity, although many of them have excellent white flesh. Filleting has been used as a common method of fish processing, however, the percentage recovery of the flesh is quite low, leaving a high amount of wastage (Bigueras, 1981). Filleting wastes, after the head and viscera are removed from the skeleton, may be over 50% of the meat (Regenstein, 1980). Despite the fact that this waste material is largely used for animal feed, it represents a significant loss of human food.

Mince fish represents a significant advance in efforts aimed at improving utilization of fish-protein human food (Murray, Stanley and Gill, 1980) and an inexpensive source of quality protein for food provided it can be incorporated into acceptable products (Daley and Deng, 1978). An important aspect of the use of minced fish is its value as raw materials in the preparation of easy to prepare fish products (Kreuzer and Dey, 1974). The market for these products is likely to increase with the expansion of use of convenience foods.

The investigation reported in this work is concerned with the utilization for human food, in the form of minced fish products, of low value pelagic fish. The minced products were developed to be suitable for marketing in both a developed and developing country, i.e., the United Kingdom and the Philippines. The possibility of typical minced fish products being marketed in the United Kingdom and other developed countries is also considered.

The study has concentrated on the nutritional, microbiological and sensory aspects of:

- (a) production of unwashed and washed minces from whole sprats,
- (b) production of fish balls using washed and unwashed minced sprat as major ingredients; and fish sausage using the same ingredients as a partial meat substitute.

2. MATERIALS AND METHODS

2.1 Fish Samples

Fresh sprats (*Sprattus sprattus*) obtained from the Grimsby Fish Docks were used in the first part of the experimental work in the manufacture of the fish ball product. Frozen samples were

utilized in the second part of the experimental work, i.e., for the manufacture of fish sausages because sprats were only available in season. This fish was chosen because it was cheap, easily available and as it closely relates to Clupeid species (*Sardinella* spp.), a low cost and abundant fish in the Philippines.

2.2 Processing of Raw Materials

Batches of 30 kg of sprats were processed as follows:

The fish samples were washed thoroughly, drained and then passed through the flesh/bone separator (Baader 696 model Nordischer Maschinenbau, Lubeck, Federal Republic of Germany) using a 3-mm perforated drum. The minced flesh was divided into two lots. The second lot was washed with tap water. Both the unwashed and washed mince were packed in low density polythene pouches and blast frozen for 3 h, then stored at -25°C ready for the product formulation (Figure 1).

2.2.1 Fish ball product

Mince fish was added as the major ingredient of this product. The dry ingredients were first mixed using a Hobart mincer and the minced sprats were then mixed in thoroughly. The resulting mixture was shaped manually in rounded forms approximately 1.5 cm in diameter.

The fish ball products were packed in laminated polyamide ionomer low density polythene, vacuum and non-vacuum packed. The packed samples were divided into two lots and stored at 4°C and -25°C. Samples stored at 4°C were tested microbiologically, chemically and by sensory evaluation for three weeks at weekly intervals. The same tests were conducted with the samples stored at -25°C fortnightly for three months (Figure 2).

2.2.2 Fish sausage product

This product utilized minced fish as a partial meat protein substitute.

The procedure followed in the sausage manufacture was patterned after the British Standard Pork Sausage and the percentage of lean pork and fat were modified accordingly. A legally permitted colouring dye, Red 2 G was added to simulate the colour of the standard pork sausages, using unwashed and washed minced sprats.

The sausages were packed in low density polythylene pouches. Storage studies were carried out on the samples with 30% incorporation for both unwashed and washed samples. An all pork sausage was made as the standard control. The samples were divided into two batches and stored at 4°C and -18°C. The samples were tested chemically, microbiologically and by sensory evaluation. The samples stored at 4°C were tested up to two weeks while the samples stored at -18°C were analysed weekly for over two months (see Figure 2).

2.3 Proximate Analysis

2.3.1 Lipid, ash, moisture and crude protein

The analysis of lipid content was carried out using the modified Bligh and Dyer (1959) method as further modified by Hanson (1980); ash content according to the methods of AOAC (1980); moisture according to the Commission of European Communities, EEC (1979) recommended method ISO R, 1442-1973, and the crude protein was determined by the EEC (1979) recommended Method ISO R 937-1969, and the method of Hanson (1982). The crude protein was calculated using a factor of 6.25.

2.3.2 Peroxide value

The peroxide value determination was carried by the method of Pearson (1976).

2.3.3 Microbiological evaluation

The total viable count and the determination of the presence of *Staphylococcus aureus* were patterned after the ICMSF (1978).

2.3.4 Sensory evaluation

- (a) **Fish balls:** Samples were served separately in polythene trays using random number codes for identification. The panelists were requested to assess the samples in accordance with the prepared taste panel score sheet.
- (b) **Sausage products:** The sausages were served in polythene trays individually using numerical codes (e.g., 929, 831) for identification. The panelists were requested to assess the samples in accordance with the parameters stated in the prepared sensory evaluation score sheet.

3. RESULTS AND DISCUSSION

3.1 General Consideration

Unviscerated sprat was used in the mince production since the evisceration of such a small fish would markedly increase the production costs of mince. Washing of the mince was, therefore, considered to be important in order to overcome some of the disadvantage of having viscera in the mince.

3.2 Proximate Analysis

The results of the proximate analysis of the fish ball samples prepared from unwashed and washed mince are given in Table 1. Results were obtained separately for batches that were to be stored differently (i.e., vacuum packed and non-vacuum packed) stored at 4°C and -25°C in order to check that the batches did not differ significantly in composition. No significant differences were found between the batches for either the unwashed or washed mince fish balls. However, the unwashed mince fish balls showed higher protein, lipid and lower moisture compared with the washed batches. This reflects the composition of the mince used. The ash content of the fish balls is higher than that of the mince by about 3.6% because of the addition of salt and the carbohydrate content of the fish balls (about 16%) results from the addition of the self-raising flour. The unwashed mince has a higher calorific value due mainly to the higher lipid content.

3.2.1 Microbiological assessment

The microbiological results given in Table 2 for samples stored at 4°C indicate that there was no significant difference between the TVCs of the vacuum packed (VP) and non-vacuum packed (NVP), both incubated at 25°C and 37°C. Bacterial numbers increased during the 21-day storage period. Psychrotrophs were presumably the organisms growing which was indicated by the more rapid increase at 25°C than 37°C. Potential health hazard organisms, coagulase positive *Staphylococci*, were not present in the samples. The ICMSF (1978) limit for comminuted fish products is 10^6 organisms per gramme. Both the unwashed mince fish balls reached this limit at about 14 days and were well above the limit by 21 days.

The results of the microbiological assessment of fish balls stored at -25°C are given in Table 3. Freezing caused a decline in the numbers of micro-organisms because the cells which are still viable after freezing gradually die off when stored in a frozen state. No coagulase positive *Staphylococci* were present in the samples. No significant difference in the TVCs of the unwashed and washed (vacuum packed and non-vacuum packed) samples was shown from 0 to 91 days of storage. The results also show that the TVCs are within the acceptable limit (10^6 /g sample) for comminuted products, set by the ICMSF (1978).

3.2.2 Fish balls stored at 4°C

Sensory evaluation results for fish balls manufactured from washed and unwashed mince, stored at 4°C for 21 days are presented in Figure 3. The significance of washing in relation to sensory parameters and pecking method are presented in Table 4 and Table 5, respectively:

- (a) **Colour:** No significant trends were observed in the sensory assessment of colour of the fish balls over the storage period. However, the washed mince fish balls had a lighter colour throughout the storage period for both vacuum and non-vacuum packed (Figure 3), and which was found to be significant at the $p < 0.01$ level (Table 4). The packaging did not have any significant effect ($p > 0.05$) on the colour for both unwashed and washed samples.
- (b) **Surface texture:** No definite trends were observed in the surface texture over the storage period. The samples from the unwashed mince had a harder texture than the samples from the washed mince and this was significant from the vacuum packed ($p < 0.01$) and highly significant for the non-vacuum packed ($p < 0.001$) (Table 4). The difference in the texture was a reflection of the starting material, i.e., the washed mince which had a high moisture content, was found to be softer than the unwashed.

No significant difference ($p > 0.05$) was found when samples were compared on the basis of different types of packaging (Table 5).

- (c) **Odour:** As seen in Figure 5 for vacuum packed and non-vacuum packed, unwashed samples increased in odour intensity with storage time, whereas the reverse trend was observed with the washed vacuum packed and non-vacuum packed. The greater odour intensity of the unwashed compared with the washed was found to be probably significant ($p > 0.05$) for both packaging methods. The packaging method itself had no significant effect on the odour.

The variation in odour intensities on storage did not appear to be reflected by similar trends in bacteriological results but could possibly be due to rancidity development or an autolytic process, both of which could be expected to be more rapid in the samples from the unwashed mince.

The results on the unwashed samples stored at 4°C, therefore, indicate that washing had a significant effect on the three sensory parameters evaluated. Whether this affects the overall consumer acceptability of the product needs to be assessed separately with appropriate consumer taste panels. However, these results clearly indicated that the two types of product (unwashed and washed) can be readily distinguished. The effect of packaging was not a major factor as no significant difference was obtained on comparing the vacuum packed with the non-vacuum packed for both the unwashed and washed samples. Microbiological quality seems to be the limiting factor in chilled storage of fish balls from both unwashed and washed mince rather than changes in sensory parameters.

3.2.3 Fish balls stored at -25°C

- (a) Colour: The results in Figure 4 indicated that the fish balls made from the washed mince were lighter in colour than those from unwashed mince. The colour scores of all the samples decreased slightly over the first three weeks then tended to increase slightly over the next ten weeks. However, the washed minced fish balls had a lighter colour throughout the storage period for both vacuum packed and non-vacuum packed, and the difference was found to be highly significant at $p < 0.001$ level (Table 6). The packaging did not have any effect on the colour for both unwashed and washed samples (Table 7).
- (b) Surface texture: No definite trend was observed on the surface texture over the storage period. The samples from the unwashed mince had a harder texture than the samples from the washed mince and this was highly significant for both vacuum packed and non-vacuum packed ($p < 0.001$) (Table 6). The difference in texture of the unwashed and washed fish balls was a reflection of the starting material, i.e., the washed mince which has a high moisture content was found to be softer than the washed. No significant difference ($p > 0.05$) was found when samples were compared on the basis of different types of packaging (Table 7).
- (c) Odour: The odour scores for both the unwashed and washed samples vacuum packed and non-vacuum packed increased over the 13th week storage period, although the increase was erratic, particularly for the washed mince fish balls, as indicated in Figure 4. On comparing the unwashed with the washed mince fish balls the odour was not significantly different from the vacuum packed and only probably significantly different ($p > 0.05$) for the non-vacuum packed samples. The odour was not affected by the packaging method (Table 7). The increase in odour could be due to auto-oxidation or the autolysis, however, washing and/or vacuum packaging would be expected to affect the rate of these processes.

The results on the unwashed samples stored at -25°C, therefore, indicated that washing had a significant effect on colour and surface texture whilst not affecting the odour to the same extent. The effect of packaging methods was not significant since no difference was obtained on comparing the vacuum packed and non-vacuum packed for both the unwashed and washed samples. Both the unwashed and washed mince fish balls were found to be microbiologically acceptable up to 91 days on storage.

3.3 Proximate Analysis

The results of the proximate analysis of the sausage products that were subsequently stored at 4° and -18°C, are given in Table 8.

The unwashed mince sausage showed higher protein, lipid, ash and lower moisture compared with washed mince sausage. This reflects the composition of the mince used. On comparing the mince sausage with the standard reference (all pork) it was observed that the mince sausages had higher protein, moisture and ash, but a lower lipid content. The calorific values of the sausages mainly reflect the lipid content, i.e., all pork greater than unwashed mince greater than washed mince sausage.

3.3.1 Microbiological assessment

The microbiological results of the samples stored at 4°C given in Table 9, indicate no significant difference in TVCs between the unwashed and washed mince sausages, but significantly lower values for all pork sausages, for both 25° and 37°C incubations. In each case the TVCs were found to increase from 0 to 14 days. Psychrotrophs appear to predominate as shown by the more rapid increase at 25°C than 37°C. Potential health hazard organisms, coagulase positive *Staphylococci* were not present in the samples.

Both the unwashed and washed mince sausages reached the IOMSF (1978) limit at about seven days, whereas the all pork was still below the limit. All the samples, however, were above the standard limit at 14 days.

The results of the microbiological assessment of the sausages stored at -18°C are given in Table 10. A decline in the number of micro-organisms due to the effect of low temperature was observed. No significant difference in the TVCs between the unwashed and washed sausages were found. However, the TVCs of the all pork sausages were significantly lower throughout the storage period.

The TVCs of all three samples were below the ICMSF limit for comminuted product of 10^6 organisms per gramme throughout the storage period (ICMSF, 1978) and the samples were found to be free from coagulase positive *Staphylococci*.

3.3.2 Sensory evaluation and peroxide value determination

3.3.2.1 Fish sausages stored at 4°C : Figure 5 gives the results of the sensory evaluation of the uncooked unwashed mince, washed mince and all pork sausages stored at 4°C for a period of 14 days. The sensory parameters evaluated were: colour, texture and odour. Table 11 indicates the significance of the washing treatment and comparison with the standard reference (all pork) in relation to the sensory parameters. Figure 6 gives the results of the peroxide value determination.

- (a) **Colour:** It is seen in Figure 5 that only small changes occur during the storage period. However, the sausages made with washed mince have lighter colour compared with those made with unwashed mince. The all pork sausage has the lightest colour among the three samples studied. The Student's *t* tests indicate that colour difference was significant ($p < 0.01$) for the unwashed mince versus washed mince sprat, unwashed versus all pork and washed versus all pork.
- (b) **Texture:** No definite trends against time were observed on the texture of the sausages over the storage period. The unwashed mince sausage had a coarser particle size than the washed mince and all pork sausages. The difference in the texture between the unwashed mince and washed mince sausages was possibly due to the washing and pressing process breaking down the particles in the mince. The Student's *t* test results in Table 12 indicate a probably significant difference ($p > 0.05$) between the unwashed and washed mince sausage and unwashed mince and all pork. On comparing the texture of washed mince sausage and all pork, no significant difference ($p > 0.05$) was obtained.
- (c) **Odour:** The odour scores of the unwashed, washed minces and all pork sausages showed an increasing trend over the storage period as indicated in Figure 5. The Student's *t* test in Table 11 indicates that the difference in odour for unwashed and washed was probably significant ($p > 0.05$) whilst the odour of the unwashed mince and all pork was highly significant ($p < 0.001$) whilst the odour of the washed mince sausage versus the all pork sausage was not significant ($p > 0.05$).
- (d) **Peroxide value:** Since the odour scores increased during the storage time, and since fish lipids are very prone to lipid auto-oxidation, peroxide values were used to assess if oxidative rancidity was developing during storage. The results are given in Figure 6. The peroxide values can be seen to increase for all three samples, with the unwashed having higher values than the washed and all pork. This is the expected order since washing is known to remove pro-oxidant compounds and lipid. Rancidity is thought to be noticeable in food products at levels between 20 and 40 mEq/kg (Pearson, 1976). This is reached more rapidly for the minced fish products but all three products have peroxide values above 20 mEq/kg by day 14.

The overall results on the uncooked samples stored at 4°C , therefore, indicate that the mince sausage products were found to be significantly different to the all pork sausage and also the washing of the fish mince had a significant effect on the three sensory parameters evaluated. How these results relate to the overall consumer acceptability of the products (which are normally sold uncooked) needs to be assessed separately with the appropriate consumer taste panels. Microbiological quality seems to be the limiting factor in the storage of sausages rather than the changes in sensory parameters, although the odour scores increase markedly over the storage period and the peroxide values indicate that rancidity may be significant after seven days storage for the mince sausages.

3.3.2.2 Fish sausages stored at -18°C : Figure 7 gives the results of the sensory evaluation of the uncooked unwashed sprat mince, washed sprat mince and all pork sausages stored at -18°C up to 70 days. Table 12 indicates the significance of the washing treatment and comparison with the all pork sausage in relation to sensory parameters. Figure 8 gives the results of the peroxide value determination.

- (a) **Colour:** Only slight changes occurred in colour during the storage period. However, the sausages made with washed mince have lighter colour among the three samples studied (Figure 8). The colour differences in the samples were found to be highly significant ($p < 0.001$).

- (b) **Texture:** No definite trends against time were observed in the texture of the sausages over the storage period. The unwashed mince had a slightly coarser texture than the washed mince, which itself had a coarser texture than the all pork sausages. The Student's t test results in Table 12 versus washed mince sausage are probably significant ($p > 0.05$), whilst highly significant ($p < 0.001$) for the unwashed mince versus all pork sausage and washed versus all pork sausage.
- (c) **Odour:** The odour scores for each of the samples showed a small increase over the storage period in contrast with the marked increase observed for the samples stored at 4°C . The odour of the unwashed mince sausage was stronger than the washed mince which was stronger than the all pork sausages (Figure 9). The Student's t tests indicate that the odour difference of the three samples was in each case highly significant ($p < 0.001$) (Table 12).
- (d) **Peroxide values:** The results of the peroxide values determination for the unwashed mince sausages, washed mince sausages and all pork sausages are shown in Figure 8. The increase in the peroxide values of the frozen sausages was not as rapid as shown by the samples stored at 4°C . Hardy (1980) pointed out that the most common method of retarding oxidation is storage at low temperature, since for every 10°C reduction the oxidation rate falls by a factor of 2 and 3.

The unwashed mince fish sausage gave higher peroxide values than the washed mince and both had higher values than the all pork sausage. As mentioned in the discussion of peroxide values of sausages stored at 4°C , this order of activity is expected. The results indicate that rancidity was not noticeable for any of the samples even at 70 days' storage. This is in agreement with the odour scores given above which show a very small increase over the storage period.

The overall results of the sensory parameters varied only slightly over the 70-day period. The odour scores showed very small increases, in agreement with the peroxide value results. The results for the uncooked samples stored at -18°C indicate that washing the sprat mince has a significant effect on the three sensory parameters evaluated. Whether this affects the overall consumer acceptability of the product (which is normally sold uncooked) needs to be assessed separately with the appropriate consumer panels. However, the results clearly indicated that the two types of product (unwashed and washed) can readily be distinguished. The mince sausages can also be readily distinguished from the all pork sausages; however, as discussed above, the products were formulated as fish sausages and not pork sausage with fish mince as an extender. The sausages were found to be microbiologically acceptable up to 70 days of storage.

4. CONCLUSION

Overall, this investigation has shown that using a Baader type flesh/bone separator, mince can be produced from whole unviscerated sprats that is microbiologically acceptable in unwashed and washed forms. Both forms of mince can be utilized in fish balls, i.e., in a product as a major ingredient, and also in fish sausages, i.e., in a product as a partial substitute for some meat protein, and in both cases the products from both the unwashed and washed mince have satisfactory shelf lives, with respect to microbiological acceptability, for both chilled and frozen storage. The study also indicated that the washed mince products, when compared with the unwashed, had sensory properties that are generally considered to be more acceptable to the consumer, particularly in developed countries where bland products are favoured. However, the washed mince has much higher production costs and this should be taken into consideration in consumer testing, particularly in developing countries.

5. REFERENCES

- AQAC (Association of Official Analytical Chemists), Official methods of analysis. Washington, D.C., 1980 AQAC, 12th ed.
- Biguera, C.M., An investigation of fish flesh yield from different species of fish using a flesh and bone separator. Post Graduate Diploma Project Report submitted to Grimsby College of Technology, U.K.
- Bligh, E.G. and W.J. Dyer, A rapid method of total lipid extraction. Can.J.Biochem.Physiol., 1959 37(8):911-7
- Commission of European Communities, REC, Recommended method 150. R Determination of protein content. Bruxelles, CEC, pp. 937-69
- _____, Recommended method 150. R Determination of moisture content. Bruxelles, CEC, 1979a pp. 1442-973

- Daley, L.H. and J.C. Dang, Determining the optimal ranges of factors affecting the sensory accept-
1978 ability of a minced mullet sausage. J.Food Sci., 43(5):1497-500
- Davidson, S., et al., Human nutrition and dietetics. London, Churchill Livingstone, pp. 12-57
1979 7th ed.
- Hanson, S.W., Determination of total lipid. Graduate course manual. Grimsby, U.K., Grimsby College
1980 of Technology, Department of Science and Food Technology
- _____, Determination of crude protein, true protein and non-protein nitrogen (Kjeldahl
1982 Method). Graduate course manual, Grimsby, U.K., Grimsby College of Technology,
Department of Science and Food Technology
- Hardy, R., Fish lipids. Part 2. In Advances in fish science and technology, edited by
1980 J.J. Connell. Farnham, Surrey, England, Fishing News (Books) Ltd., pp. 103-11
- ICMSF (International Commission of Microbiological Standards of Foods), Microorganisms in foods.
1978 2. Toronto, Canada, University of Toronto Press, pp. 92-104
- Kreuzer, R. and C. Day, Need for product development and technological advances in utilising fish-
1974 ery resources. In Fishery products, edited by R. Kreuzer. West Byfleet, Surrey,
England, Fishing News (Books) Ltd., for FAO, pp. 278-82
- Murdoch, J. and J.A. Barnes, Statistical tables. London, Macmillan, 2nd ed.
1974
- Murray, G.F., D.W. Stanley and T.A. Cill, Improved utilisation of fish protein-co-extrusion of
1980 mechanically deboned salted minced fish. Can.Inst.Food Sci.Technol.J., 13(3):125-30
- Pearson, D., The chemical analysis of foods. London, Churchill Livingstone, pp. 494-6. 7th ed.
1976
- Regenstein, J.M., The Cornell experience with minced fish. In Advances in fish science and
1980 technology, edited by J.J. Connell. Farnham, Surrey, England, Fishing News (Books) Ltd.,
pp. 192-8
- Robertson, J.A., The sprat and the sprat fishery in England. Fish.Invest.Minist.Agric.Fish.Food
1938 C.B.(2 Sea Fish.), 16(2)
- Wallyn, A., Separation of flesh and bone by the Paoli separator. In Proceedings of the Conference
1976 on the production and utilisation of mechanically recovered fish flesh (minced fish),
edited by J.N. Keay. Aberdeen, MAFF Torry Research Station, pp. 29-30
- Wells, L.A., The observers book of sea fishers. London, Frederick Warr and Co. Ltd., pp. 59-65
1958

Table 1
Proximate analysis of uncooked fish balls

Sample	Crude protein	Mean percentage composition ^{a/}			Carbohydrate value (by difference)	Calorific value (kcal/100 g)
		Lipid	Moisture	Ash		
A. Unwashed mince fish balls						
(1) Vacuum packed (a) Stored at 4°C	13.9 (0.1)	11.3 (0.5)	55.1 (0.4)	5.1 (0.1)	14.6	216
(b) Stored at -25°C	14.1 (0.0)	10.4 (0.0)	54.8 (0.4)	4.9 (0.4)	15.8	213
(2) Non-vacuum packed						
(a) Stored at 4°C	13.9 (0.0)	10.0 (0.2)	56.1 (0.4)	5.2 (0.0)	14.8	205
(b) Stored at -25°C	14.1 (0.0)	9.9 (0.1)	54.5 (0.8)	5.0 (0.2)	16.5	212
B. Washed mince fish balls						
(1) Vacuum packed (a) Stored at 4°C	9.1 (0.7)	6.6 (0.0)	63.3 (0.3)	4.4 (0.1)	16.6	162
(b) Stored at -25°C	10.1 (0.0)	7.0 (0.0)	60.6 (0.4)	4.2 (0.3)	18.1	176
(2) Non-vacuum packed						
(a) Stored at 4°C	9.1 (0.7)	7.1 (0.4)	61.7 (0.6)	4.3 (0.1)	17.8	172
(b) Stored at -25°C	10.0 (0.0)	6.4 (0.6)	58.4 (0.5)	4.3 (0.1)	20.9	181

^{a/} Mean of four determinations. Figures in brackets are standard deviations

^{b/} Calculated values using factors of 9 kcal/g for lipid, 4 kcal/g for protein and 4 kcal/g for carbohydrate (Davidson *et al.*, 1979)

Table 2
Microbiological assessment of uncooked fish balls stored at 4°C

Unwashed mince fish balls						
Storage time Days	Total Viable Counts per gram of sample			Coagulase positive <i>Staphylococci</i>		
	25°C	37°C		25°C	37°C	
	VP	NVP	VP	NVP	VP	NVP
0	6.1x10 ⁴	6.7x10 ⁴	6.0x10 ³	7.5x10 ³	-ve	-ve
7	2.8x10 ⁵	2.5x10 ⁵	5.2x10 ⁴	6.3x10 ⁴	-ve	-ve
14	1.3x10 ⁶	1.5x10 ⁶	4.8x10 ⁵	5.2x10 ⁵	-ve	-ve
21	5.5x10 ⁷	5.2x10 ⁷	4.5x10 ⁶	5.1x10 ⁶	-ve	-ve

Washed mince fish balls						
Storage time Days	Total Viable Counts per gram of sample			Coagulase positive <i>Staphylococci</i>		
	25°C	37°C		25°C	37°C	
	VP	NVP	VP	NVP	VP	NVP
0	5.7x10 ⁴	5.1x10 ⁴	5.4x10 ³	5.0x10 ³	-ve	-ve
7	2.5x10 ⁵	2.2x10 ⁵	3 x10 ⁴	3.5x10 ⁴	-ve	-ve
14	1.2x10 ⁶	1.4x10 ⁶	4.2x10 ⁵	4.5x10 ⁵	-ve	-ve
21	4.9x10 ⁷	5.0x10 ⁷	4.4x10 ⁶	4.9x10 ⁶	-ve	-ve

VP = Vacuum packed
NVP = Non-vacuum packed

Table 3

Microbiological assessment of uncooked fish balls stored at -25°C

Unwashed mince fish balls					
Storage time Days	Total Viable Counts per gram of sample				Coagulase positive <u>Staphylococci</u>
	25°C		37°C		
	VP	NVP	VP	NVP	
0	6.1x10 ⁴	6.7x10 ⁴	6.0x10 ³	7.5x10 ³	-ve
21	5.8x10 ³	6.1x10 ³	5.5x10 ²	6.2x10 ²	-ve
35	4.7x10 ³	5.7x10 ³	4.9x10 ²	5.8x10 ²	-ve
49	3.9x10 ³	5.2x10 ³	3.8x10 ²	4.6x10 ²	-ve
63	3.7x10 ³	4.8x10 ³	3.3x10 ²	4.2x10 ²	-ve
77	3.3x10 ²	4.3x10 ²	2.8x10 ²	3.5x10 ²	-ve
91	2.9x10 ²	3.5x10 ²	2.4x10 ²	3.2x10 ²	-ve
Washed mince fish balls					
Storage time Days	Total Viable Counts per gram of sample				Coagulase positive <u>Staphylococci</u>
	25°C		37°C		
	VP	NVP	VP	NVP	
0	5.1x10 ⁴	5.7x10 ⁴	5.4x10 ³	5.0x10 ³	-ve
21	5.0x10 ³	5.2x10 ³	4.8x10 ²	4.9x10 ²	-ve
35	4.8x10 ³	4.9x10 ³	4.4x10 ²	4.2x10 ²	-ve
49	3.9x10 ³	4.3x10 ³	4.1x10 ²	4.1x10 ²	-ve
63	3.5x10 ³	4.1x10 ³	3.8x10 ²	3.9x10 ²	-ve
77	3.2x10 ²	3.5x10 ²	3.2x10 ²	3.4x10 ²	-ve
91	2.5x10 ²	2.9x10 ²	2.7x10 ²	2.9x10 ²	-ve

Table 4

The significance of washing on the sensory parameters of uncooked fish balls stored at 4°C
(Values of Student's t and levels of significance of difference in scores
- at 0, 7, 14 and 21 days - for unwashed versus washed samples)

Sensory parameter and packaging	Values of t and levels of significance ^{a/}		$\gamma = 3$ degrees of freedom	
	Not significant > 0.05	Probably significant < 0.05-0.01	Significant < 0.01-0.001	Highly significant < 0.001
(a) <u>Colour</u>				
VP	-	-	7.55	-
NVP	-	-	10.00	-
(b) <u>Surface texture</u>				
VP	-	-	7.56	-
NVP	-	-	-	15.39
(c) <u>Odour</u>				
VP	-	3.64	-	-
NVP	-	3.70	-	-

a/ Levels of significance and degree of freedom from Murdoch and Barnes (1974)

Table 5

The significance of packaging method on the sensory parameters
of uncooked fish balls stored at 4°C
(Values of Student's t and levels of significance of differences in scores
- at 0, 7, 14 and 21 days - for vacuum packed versus non-vacuum packed samples)

Sensory parameter and washing treatment	Values of t and levels of significance ^{a/}		$\gamma = 3$ degrees of freedom	
	Not significant > 0.05	Probably significant < 0.05-0.01	Significant < 0.01-0.001	Highly significant < 0.001
(a) <u>Colour</u>				
Unwashed	0.97	-	-	-
Washed	1.11	-	-	-
(b) <u>Surface texture</u>				
Unwashed	1.31	-	-	-
Washed	0.63	-	-	-
(c) <u>Odour</u>				
Unwashed	0.29	-	-	-
Washed	0.86	-	-	-

a/ Levels of significance and degrees of freedom from Murdoch and Barnes (1974)

Table 6

The significance of washing on the sensory parameters of uncooked fish balls stored at -25°C
(Values of Student's t and levels of significance of differences in scores - at 0-91 days -
for unwashed versus washed samples)

Sensory parameter and packaging	Values of t and levels of significance ^{a/}		$\gamma = 6$ degrees of freedom	
	Not significant > 0.05	Probably significant < 0.05-0.01	Significant < 0.01-0.001	Highly significant < 0.001
(a) <u>Colour</u>				
VP	-	-	-	10.71
MVP	-	-	-	13.83
(b) <u>Surface texture</u>				
VP	-	-	-	6.43
MVP	-	-	-	8.33
(c) <u>Odour</u>				
VP	1.70	-	-	-
MVP	-	2.36	-	-

a/ Levels of significance and degrees of freedom from Murdoch and Barnes (1974)

Table 7

The significance of packaging method on the sensory parameters of uncooked fish balls
stored at -25°C

(Values of Student's t and levels of significance of differences in scores
- at 0-91 days - for vacuum packed versus non-vacuum packed samples)

Sensory parameter and washing treatment	Values of t and levels of significance ^{a/}		$\gamma = 6$ degrees of freedom	
	Not significant > 0.05	Probably significant < 0.05-0.01	Significant < 0.01-0.001	Highly significant < 0.001
(a) <u>Colour</u>				
Unwashed	1.32	-	-	-
Washed	0.34	-	-	-
(b) <u>Surface texture</u>				
Unwashed	2.36	-	-	-
Washed	0.19	-	-	-
(c) <u>Odour</u>				
Unwashed	0.77	-	-	-
Washed	0.27	-	-	-

a/ Levels of significance and degrees of freedom from Murdoch and Barnes (1974)

Table 8

Proximate analysis of formulated sausages

Sample	Crude protein	Lipid	Mean percentages ^{a/}		Carbohydrate (by difference)	Calorific ^{b/} values kcal/100 g
			Moisture	Ash		
Unwashed mince sausage	16.8 (0.1)	13.3 (0.2)	54 (0.2)	2.2 (0.1)	13.7	242
Washed mince sausage	13.3 (0.1)	10.2 (0.1)	58 (0.3)	1.91 (0.6)	16.6	211
All pork sausage	10.8 (0.1)	21.2 (0.6)	48 (0.3)	1.77 (0.1)	18.2	307

^{a/} Mean of four determinations. Figures in brackets are standard deviations^{b/} Calculated values using factors of 9 kcal/g for lipid, 4 kcal/g for protein and 4 kcal/g for carbohydrate (Davidson *et al.*, 1979)

Table 9

Microbiological assessment of sausages stored at 4°C

Storage time (days)	Unwashed	Total viable counts per gramme of sample					Coagulase positive <i>Staphylococci</i>
		Washed (25°C)	All pork	Unwashed	Washed (37°C)	All pork	
0	4.5x10 ⁵	3.5x10 ⁵	3.8x10 ⁴	3.5x10 ⁴	3.2x10 ⁴	4.4x10 ³	-ve
7	1.1x10 ⁶	1.0x10 ⁶	2.5x10 ⁵	2.9x10 ⁵	2.5x10 ⁵	2.3x10 ⁴	-ve
14	5.7x10 ⁷	4.3x10 ⁷	3.2x10 ⁶	5.5x10 ⁶	5.3x10 ⁶	5.3x10 ⁶	-ve

Table 10

Microbiological assessment of sausages stored at -18°C

Storage time (days)	Unwashed	Total viable counts per gramme of sample					Coagulase positive <i>Staphylococci</i>
		Washed (25°C)	All pork	Unwashed	Washed (37°C)	All pork	
0	4.5x10 ⁵	3.5x10 ⁵	3.8x10 ⁴	3.5x10 ⁴	3.2x10 ⁴	4.4x10 ³	-ve
7	5.4x10 ⁴	4.8x10 ⁴	4.5x10 ³	5.2x10 ³	5 x10 ³	4.8x10 ²	-ve
14	5.2x10 ⁴	4.6x10 ⁴	5.4x10 ³	4.9x10 ³	4 x10 ³	4.7x10 ²	-ve
21	5 x10 ³	4.5x10 ³	5 x10 ²	5.3x10 ²	4.8x10 ²	4.3x10 ²	-ve
28	4.8x10 ³	4 x10 ³	4.5x10 ²	5.3x10 ²	4.6x10 ²	3.9x10 ²	-ve
35	4.5x10 ³	3.9x10 ³	4 x10 ²	4.8x10 ²	4.2x10 ²	3.5x10 ²	-ve
42	4.2x10 ³	3.6x10 ³	3.6x10 ²	4.6x10 ²	3.8x10 ²	3.3x10 ²	-ve
49	3.8x10 ²	3.2x10 ²	3.2x10 ²	3.7x10 ²	3.7x10 ²	3.5x10 ²	-ve
56	3.4x10 ²	2.7x10 ²	2.8x10 ¹	3.5x10 ²	3.3x10 ²	2.7x10 ²	-ve
63	3.4x10 ²	2.6x10 ²	2.6x10 ¹	3.3x10 ²	3.6x10 ²	2.5x10 ¹	-ve
70	2.8x10 ²	2.4x10 ²	2.4x10 ¹	2.9x10 ²	2.5x10 ²	2.3x10 ¹	-ve

Table 11

The significance of washing on the sensory parameters of uncooked sausages stored at 4°C
(Values of Student's t and levels of significance of differences in scores
- at 0, 7 and 14 days - for unwashed, washed and all pork sausages)^{a/}

Parameter	Values of t and levels of significance ^{a/}		γ = 2 degrees of freedom	
	Not significant > 0.05	Probably significant < 0.05-0.01	Significant < 0.01-0.001	Highly significant < 0.001
<u>Colour</u>				
Unwashed vs. washed	-	-	8.98	-
Unwashed vs. all pork	-	-	15.64	-
Washed vs. all pork	-	-	8.98	-
<u>Texture</u>				
Unwashed vs. washed	-	2.66	-	-
Unwashed vs. all pork	-	3.04	-	-
Washed vs. all pork	2.26	-	-	-
<u>Odour</u>				
Unwashed vs. washed	-	3.38	-	-
Unwashed vs. all pork	-	-	5.97	-
Washed vs. all pork	1.71	-	-	-

^{a/} Levels of significance and degrees of freedom from Murdoch and Barnes (1974)

Table 12

The significance of washing on the sensory parameters of uncooked sausages stored at -18°C
(Values of Student's t and levels of significance of differences in scores - at 0-70 days -
for unwashed, washed and all pork sausages)^{a/}

Parameter	Values of t and levels of significance ^{a/}		γ = 10 degrees of freedom	
	Not significant > 0.05	Probably significant < 0.05-0.01	Significant < 0.01-0.001	Highly significant < 0.001
<u>Colour</u>				
Unwashed vs. washed	-	-	-	13.93
Unwashed vs. all pork	-	-	-	32.46
Washed vs. all pork	-	-	-	21.56
<u>Texture</u>				
Unwashed vs. washed	-	3.55	-	-
Washed vs. all pork	-	-	-	10.99
Washed vs. all pork	-	-	-	8.54
<u>Odour</u>				
Unwashed vs. washed	-	-	-	9.75
Unwashed vs. all pork	-	-	-	17.95
Washed vs. all pork	-	-	-	9.95

^{a/} Levels of significance and degrees of freedom from Murdoch and Barnes (1974)

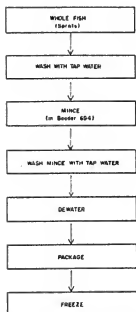


Figure 1 Flow diagram of mincing

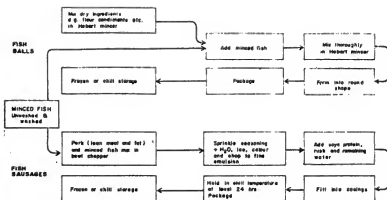


Figure 2 Flow diagram of fish balls and fish sausage manufacture

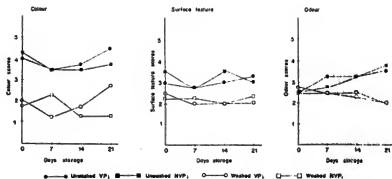


Figure 3 Variation in sensory parameters for uncooked mince fish balls stored at 4°C

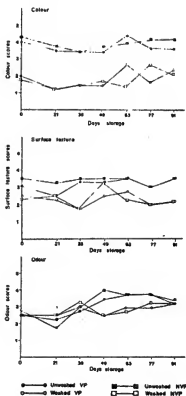


Figure 4 Variation in sensory parameters for uncooked mince fish balls stored at -25°C

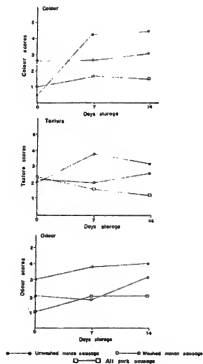


Figure 5 Variation in sensory parameters for uncooked sausage stored at 4°C

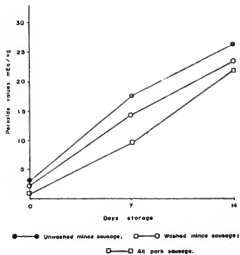


Figure 6 Peroxide values of uncooked sausages stored at 4°C

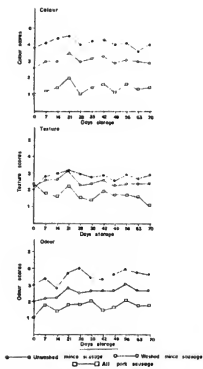


Figure 7 Variation in sensory parameters for uncooked sausages stored at -18°C

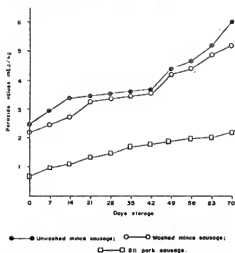


Figure 8 Peroxide values of uncooked sausages stored at -18°C

JOINT PROJECT ON FISH-DRYING IN EAST JAVA, INDONESIA

by

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ABSTRACT

This Project commenced in July 1984 and will run for a three-year period with support from ACIAR. The organizations involved are:

- (a) Research Institute of Fishery Technology, Jakarta.
Agency for Agricultural Research and Development
- (b) University of Brawijaya, Malang, Indonesia
- (c) School of Food Technology, University of New South Wales/
School of Agriculture, La Trobe University

Experimental procedure has been divided into seven areas with various organizations responsible for each area.

- Area 1 - Onboard Handling (RIFT)
- Area 2 - Fish Landings and Classification (RIFT)
- Area 3 - Salting and Brining (UNIBRAW)
- Area 4 - Drying at Muncar (UNIBRAW)
- Area 5 - Fundamental Drying Studies (UNSW)
- Area 6 - Evaluation of Dried Products (UNIBRAW/RIFT)
- Area 7 - Packaging, Storage, Transport, Distribution, Marketing Retail (RIFT/La Trobe)

The Project aims to reduce post-harvest losses through the production of traditional products by improved practices that find acceptance in both economic and aesthetic terms by the Indonesian people.

As this Project commenced in June 1984, the aim of this paper is to present the background and objectives of the Project.

Indonesia is a major fishing nation currently harvesting 1.6 million tons annually with the potential to markedly increase this catch with the subsequent aim of doubling the per capita consumption of fish by Indonesians. It was realized that present patterns of post-harvest fish losses due to spoilage would negate any increase in catch, therefore, this pattern of loss needed to be reversed.

More than 50% of the Indonesian catch is processed by traditional practices and dried-salted fish represents a proportion of these traditional products. Post-harvest losses of dried fish as a result of spoilage have been estimated at approximately 30%.

This Project aims to reduce post-harvest losses of dried fish through improved practices from the time of capture to the time of retail sale. A complete economic evaluation will be undertaken on all stages of this study.

The Project is jointly supported by the Indonesian Government through the Research Institute of Fishery Technology (RIFT), the Australian Government through the Australian Centre for International Agricultural Research/University of New South Wales/La Trobe University and the Canadian Government through the International Development Research Centre/University of Brawijaya, East Java. In an attempt to limit the scope of the Project to a manageable level it was decided to focus the study on one major fishing village in East Java. The village chosen is Muncar which is situated on the Bali Straits - Muncar was chosen because it represents a microcosm of traditional Indonesian fish capture and processing practices.

One of the main features of the Project is the manner in which all the phases of fish capture, processing and marketing have been integrated in a single study in recognition of the fact that the current spoilage problems associated with dried fish can be attributed to failings in the handling, packaging, storage and transport of dried fish equally as much as inadequacies in current processing and drying practices.

The study has been divided into four individual sections with a participating organization responsible for the execution of each section:

- (1) RIFT - Onboard Handling
- (2) RIFT - Fish Handling and Classification
- (3) UNIBRAW/UNSW - Salting and Drying
- (4) RIFT/La Trobe/UNIBRAW - Distribution, Marketing and Retail

Every six months a coordination committee, made up of representatives of participating organizations, meets to discuss Project results and to plan the following six months' work in an effort to maximize cooperation between organizations.

The experimental procedure has been divided into seven distinct areas and a brief description of each area is as follows:

Area 1 - Onboard Handling (RIFT)

- (1) Need to define existing traditional practices.
- (2) Cost/benefit analysis of various onboard practices including chilled seawater, icing and salt.

Area 2 - Fish-Landing and Classification (RIFT)

- (1) For traditional practices need to determine tonnage, usage patterns and price of major species as well as sensory, chemical and microbiological grading factors.
- (2) By experiment, will establish a five-tier grading scale and confirm scale using chemical and microbiological parameters. Also identify grades suitable for producing dried fish.

Area 3 - Salting/Brining (UNIBRAW)

- (1) Define current traditional practices, i.e., salt concentration, time, sanitation.
- (2) Experimentally assess benefits of variation in salt concentration, time, pH on the five grades of fresh fish identified in Area 2.

Area 4 - Drying at Muncar (UNIBRAW)

- (1) Define current drying methods in terms of time, A_w , moisture content and salt concentration. Also identify problem areas.
- (2) Assess the mechanical dryer installed at Muncar by UNIBRAW/IDRC. Much of this work has already been done by UNIBRAW staff, but need to examine fish quality in terms of dryer-operating conditions.

Area 5 - Fundamental Drying Studies (UNSW)

Experimentally establish fish-drying curves in terms of air temperature, velocity and humidity as well as product orientation, fatty and non-fatty fish, pre-treatments and fish shape and size. Also assess product quality and solve problems found in the Muncar dryer.

Area 6 - Evaluation of Dried Products (UNIBRAW/RIFT)

- (1) Define current traditional products in sensory, chemical and microbiological terms; also examine storage life as determined by chemical indices and sensory acceptance factors.
- (2) Establish five quality categories of dried fish and relate pre-drying categories to post-drying categories. Examine shelf life of experimentally dried fish as determined by chemical indices and sensory acceptance.

Area 7 - Packaging, Storage, Transport, Marketing and Retail (RIFT/La Trobe)

Comparative study of traditionally and experimentally produced dried fish:

- identify problem areas;
- assess wastage due to existing conditions;
- price and acceptability at all stages of capture, processing and marketing;
- compare sensory, chemical and microbiological indices for traditional and experimental products;
- intense economic study in an attempt to justify the use of mechanical drying at village level. This study will take the form of cost/benefit analyses of procedures developed to overcome defects in traditional practices.

The Project will ultimately produce the following results:

- a definition of the extent and cause of traditional dried fish wastage from capture to retail sale;
- the definition and implementation of process improvements that overcome this wastage, including the cost/benefit of the improvements;
- establish in Muncar a model dried-fish production unit, operated by the village cooperative, with the aim of extending the process to other regions in Indonesia.

Although we have only been working on this Project for five months a considerable amount has already been achieved. The groups involved in the Project are working on several areas simultaneously and are planning experiments that are both technical and economic in aspect.

In Indonesia onboard handling experiments have begun. These experiments are comparing chilled seawater storage of fresh fish with traditional ambient storage. Additionally, details of fish quality and price at the Muncar auction are being recorded on a daily basis in an effort to establish the effect of storage temperature on fish quality and consequently fish price. A small laboratory is to be established at Muncar in cooperation with a local fish cannery. The laboratory will be staffed by RIFT scientists and will undertake chemical and microbiological analyses of fresh and dried fish samples taken in the Muncar region.

In Australia the design of an experimental dryer is at an advanced stage. The dryer will be located at the School of Food Technology, University of New South Wales, and has been designed with maximum flexibility of use in mind. The unit will incorporate sophisticated data acquisition and programmable control equipment in order that all relevant data on dryer operation and product response to predetermined computer controlled drying conditions is recorded for close scrutiny at the conclusion of drying. A system has been developed to weigh the product continuously *in situ* with an accuracy of 0.1 g and these readings are fed for storage into our computer. From this data we will be able to monitor closely moisture loss as a function of a particular drying regime. A laboratory has also been renovated and committed to this Project and it will be here that fresh and dried fish will be analysed chemically and microbiologically.

In conclusion, a lot of planning has gone into this Project by all the organizations involved to ensure that the aims of the Project are met and principally that all useful improvements to current practices flow on to the people who rely on dried fish for a livelihood and for sustenance.

AN OVERVIEW OF HANDLING AND ICING RESEARCH
IN THE PHILIPPINES

by

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ABSTRACT

The paper summarizes research work on handling and icing of fresh fish in the Philippines between 1965 and 1984. Species studied include shrimps, mussels, milkfish, roundsad and Faughn's mackerel.

The Philippines is a tropical country consisting of more than 7 100 islands. The territorial open sea and inland waters are estimated to be more than five times the total land area (BFAR, 1980). This makes it a very large fishing ground, thus fish is considered one of the most important protein sources of the country. Despite the wide rich fishing ground and an increasing fisheries production, there is still a large gap between available fish protein to meet the needs of the people. Post-harvest losses are estimated to be 20-40% of the total fisheries production which amounts to about 0.4 t annually. This problem can be traced to the following:

- (1) poor handling and icing practices;
- (2) unavailability of ice and appropriate containers and insulated fish transport to rural fish-landing areas;
- (3) lack of quality consciousness on the part of the fishermen and processors, as well as the consumers; and
- (4) insufficient knowledge of fish processing technology within the fishing market chain.

Another important factor contributing to the items listed above is the fact that 20 years ago, the emphasis of research on post-harvest technology of fish concentrated more on processing aspects. Upon examination of available literature, progress reports, undergraduate and masteral theses from recognized institutions, journals and popular publications during the past 15 years, only eight meaningful research studies were gathered. The details of these are presented in Table 1. Only four species of fish and shellfish were subjects of a comprehensive and in-depth study consisting of microbiological and biochemical changes as correlated with sensory changes. These are roundsad (*Demigasterus rufus*), Faughn's mackerel (*Rastrelliger faughni* Matsui), milkfish (*Chanos chanos*) and prawns (*Penaeus monodon*). The research highlights on milkfish consist of studies on the effects of pre-chilling and icing during transportation by air, sea or land; containers used in freezing of milkfish and the standardization of the recommended processes. Four types of containers for transportation by sea were used in this study (Dolendo, 1978):

Type A - one-cubic-metre container made from wood of 1 in thick and 6.0 in wide with removable covers and locks.

B - the same size as Type A, but the interior lined with plain galvanized iron sheet on all sides.

C - an innovation of Type B. One-inch thick styrofoam alebs were placed between the plain GI sheet and wood on four sides, including the bottom and cover. Four outlets of 1 1/2 in diameter of GI drain pipes on four sides at the bottom were installed to serve as drainage for melting ice.

D - an innovation of Type C. The inner compartment of Type C was divided into three horizontal layers with movable wooden boards resting on the bottom. The four banners were provided with six holes of 1/2 in diameter as drainage for melted ice. The cover of the box was placed at the side perpendicular to the bottom and not at the top so that the banners can be removed without removing the wooden board.

Styrophore boxes and bañeras were used in handling and transporting by air and land, respectively. The quality assessment was primarily based on the Food Terminal Inc., (FTI) Standards.

The recommendations of the study are (Dolendo, 1978):

- (1) Pre-chilling of the milkfish to 4°C right after harvest is a very important step to preserve the quality during transport. For every ton of fish, 450 kg ice with water is required to bring the temperature of the fish to 4°C within 2 h.
- (2) Type of container:
 - (a) Air transport - styrophore boxes are ideal with 1:4 ratio of ice to fish for longer distances and 1:10 to no icing for shorter distances.
 - (b) Water transport - Type D container with 1:10 ratio of ice to fish.
 - (c) Land transport - Bañeras with 1:1 ratio of ice to fish for 3-h travel and 1:2 for 1.5-h travel.

The results of the work on round scad, mackerel and prawns are presented in these proceedings by Barile et al., and Reilly, Bernarte and Dangle (1984).

There are certain gaps identified upon analysis of works listed in Table 1. These are:

- (1) in-depth microbiological and biochemical studies of milkfish;
- (2) mesophilic spoilage of milkfish;
- (3) effects of delay in icing of milkfish; and
- (4) in-depth studies on handling of mussels.

In addition, there are about 10 commercially important species that must be studied, e.g., jacks, scads, tuna and others.

Typical fish handling practices in the Philippines include poor standards of hygiene, storage at ambient temperature, use of non-insulated containers for transport and storage. From the moment fish is caught, it is usually left for 2-4 h with little or no ice until it is bought. Fish, especially large-bodied species, are usually displayed for hours in the markets without icing, such that its temperature is above 20°C.

With these gaps already identified, the Department of Fish Processing, University of the Philippines in the Visayas will contribute its humble share to the improvement of post-harvest handling in the Philippines through its active participation in the conduct of comprehensive research studies on handling, delay in icing and mesophilic spoilage of commercially important species of fish. Solid findings will be disseminated in simplified form to the industry and fishermen.

REFERENCES

- BFAR (Bureau of Fisheries and Aquatic Resources), Fisheries statistics of the Philippines. Manila, 1980 BFAR
- Bersamin, S.V. and E.P. Tongco, Preliminary studies on temperature assessment of fish at all stages 1971 in the distribution chain. Philipp.J.Fish., 9(1-2):38-44
- Dolendo, A.L., et al., Standardisation of handling, icing and freezing of milkfish. In Milkfish 1978 (Bangos) as Food. Manila, National Science Development Board, pp. 40-73
- Guevarra, G., et al., Studies on handling and depuration of green bay mussels. Philipp.J.Fish., 1978 16(2):105-16
- Legaspi, A.S. and Yeo Wee Khiong, Comparative studies on the keeping quality of cooked, beheaded 1972 and whole shrimps stored in ice. Philipp.J.Fish., 10(1-2):119-31
- Reilly, A., M.A. Bernarte and E. Dangle, Storage stability of brackishwater prawns during processing for export. Food Technol.Aust., 36(6):283-6

Table 1

Summary of handling and icing research studies in the Philippines for the past 15 years

Species Studied	Study	Findings	Recommendations	Author(s)
Assorted species	Preliminary studies on temperature assessment of fish at all stages of distribution	To conduct a survey on temperature of fish caught by hand and purse seines until marketed	Fish handling practices required improvement. Use of insulated and standardized fish containers with provisions for drainage insured proper preservation of the catch and stabilize prices	S.V. Bersamin, 1971 (Aug.-Oct., 1965)
Shrimp	Comparative studies on the keeping quality of cooked, beheaded and whole shrimps stored in ice	To determine the effects of pre-processing on the keeping quality of shrimp stored in ice	Point of rejection of shrimp: 16 days - cooked 14 days - raw, beheaded 11 days - raw, whole	A.S. Legaspi, 1972
Milkfish	Standardization of handling, icing and freezing of milkfish	To study the chilling, ice-to-fish ratios, suitable containers and modes of transport	Pre-chilling to 4°C was necessary for milkfish. For air transport: use of styrofoam 1:4 ice to fish for long distances. 1:10 to no icing for short distances. For sea transport: Type D container with 1:10 ice-fish ratio. For land transport: bananas with ratio of ice-to-fish, 1:10 3-h travel; 1:2 1½-h travel	A. Dolendo, 1978
Green bay mussels	Studies on the handling and depuration of green bay mussels (<i>Perna perna</i>)	To prolong life of mussels out of water using different methods. To determine effects of depuration on microbial load	Mussels were kept alive out of water for 4 days at 16° + 20°C. Depuration using clean seawater, 3% iodized salt solution, 3% corras salt solution and tapwater was found to be effective	G. Guavarrá, et al., 1978
Round scad and Faughn's mackerel (Alumahan)	Improvement of post-harvest handling and chilling of round scad and alumahan	To establish optimum conditions for handling and chilling of round scad and alumahan. To establish the optimum conditions for handling and chilling tilapia prior to transport. To develop suitable containers for transport of round scad and alumahan		A. Villadsen, et al., 1981-84 (Unpublished progress reports)

Table 1 (continued)

Species Studied				
Brackishwater prawn	Storage stability of brackishwater prawns during processing for export	To determine the level and type of microbial population of <i>P. monodon</i> from brackishwater ponds. To evaluate how this microbial load varied during processing. To establish codes of practice for prawn processing. To determine effective levels of sodium metabisulphite to inhibit metamorphosis during ice storage	Microbial load of brackishwater prawn is low compared with marine species. There is risk that enteric pathogens may be present when untreated chicken manure is used as fertilizer. Prawns can be stored on ice for 5 days without serious losses in quality. Treatment with $\text{Na}_2\text{S}_2\text{O}_5$ (5 g/L) can extend shelf life in ice to 10 days without melanosis. TMA and TVN are not good indices of quality. X-value of 20-30% can be used as freshness index	Railly, Bernarta and Bangia, 1984
Round scad	Improved handling in municipal fisheries	To investigate existing handling and distribution practices of fish and other aquatic products. To alleviate current problems in fish handling and distribution by conducting applied research that will aim to reduce cost and waste. To make cost-benefit analysis of fish boxes used in the area and recommend the most suitable for their purpose. To educate the municipal fishermen on proper handling and distribution methods by conducting short-term training programs. To work toward achieving an integrated approach to the development of the pilot area	Observational: (Site-Navotas, Cavite, Batangas, Misican and Ilcos Sur) Chilling of catch by use of ice in certain localities. Few used chilled seawater (CSW). Icing during auction was not common. Containers were usually piled one on top of another causing damage to fish. Styrofoam boxes were better than other fish containers but the use of reinforced styrofoam box was more advantageous. Instructional material/simplified brochures were printed for extension services. Use of CSW system. Maintained comparative quality to ice fish but methods should be improved	J. Paralta and F. Orjans, 1984 (unpublished terminal report)



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